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THE STOMACH SPIROCHETE OCCURRING IN MAMMALS

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In 1893 Bizzozero discovered a spirochete in the stomach of dogs in Italy which he stated was in the parietal cells of the peptic glands. Salomon (1896), examining various animals in Germany, detected the same organism in the stomach of dogs, cats and rats, and was able to transmit it by feeding to the stomach of the mouse. He distinguished three forms morphologically and also found the spirochete to be actively motile with terminal flagella. Balfour (1906), in Egypt, observed spirochetes in gastric and intestinal ulcers of dogs and monkeys, produced by inoculation with a trypanosome (*Trypanosoma dimorphon?*). In the same year Krienitz (1906), in Germany, found three forms of spirochetes in the fresh stomach contents of a patient suffering from stomach cancer and later (1906a) studied the morphological changes resulting from alternations in environment. Afterward Regaud (1909), in France, found the organism by means of darkfield illumination and proved it to be a living micro-organism, notwithstanding that Carnot and Lelièvre (1909) had described it as the secretion product of parietal cells. In the following year Lucet (1910) detected two forms of spirochete in lesions of a dog suffering from hemorrhagic gastro-enteritis. Ball and Roquet (1911), however, regarded this spirochete as identical with that described by Regaud and called it *Spirochaeta regaudi.* They stated, moreover, that this spirochete occurring in the normal stomach of dogs has probably no causative relation to hemorrhagic gastro-enteritis. Suda (1916), in Japan, also observed spirochetes in the gastric gland of dogs.

The present paper is a report of investigations undertaken in connection with our encountering this spirochete in the stomach of a rat in the course of an examination of the alimentary tract (1917).

Morphology.—The stomach spirochete is an inflexible spiral with a straight longitudinal axis, the transverse section being an ellipse, and is provided with one short, thin flagellum at each end. In the stained preparation, the most common type of this spirochete (Fig. 1) is a fine form measuring from 4.5 to 10.5μ and the number of turns is 4 or 5 to

14 or 15. Every two consecutive turns are closely set, the distances between these pairs of spirals being regular. There was frequently seen, however, in the stomach of the cat and monkey a form somewhat shorter, with a wider spiral and a more acute turn (Fig. 2). Another form (Fig. 3), usually occurring in the stomach of the dog, is stout, and the turns are shallower and fewer. Such a form is especially observed in the stomach of the rabbit, and also often of the mouse and guinea-pig, if it be transmitted.

Thus, like Salomon, we were able to distinguish three forms morphologically, although our classification is slightly different. It is uncertain whether these forms represent in reality three different species or belong to one species with temporary variations in form. The degenerated spirochete frequently assumes the spiral body curved or twisted, or with irregularly relaxed turns. There are also seen individuals faintly or heterogeneously stained, and even in "moniliform degeneration" (Fig. 1). Moreover, in preparations stained with Giemsa's stain, we can very often detect so-called "involution forms," which show from one to five or more chromatin-like granules stained intensely red at the outer ends of turns, notwithstanding the faint staining of the body. Such granules also are occasionally detected arranged along the main axis in the rod-shaped spirochete (Fig. 3).

This spirochete in the supravital staining is not found differentiated from that of the foregoing description.

The spirochete in the dark-field illumination shows a fair distinction (Fig. 4). The consecutive turns seem almost to touch, and accordingly the whole body presents the appearance of a coil, the transverse section of which was clearly proved by the rotatory movement of the organism to be an ellipse. The dark-field microscopic view also shows that each of the two extremities of this spirochete is tapered into a fine terminal flagellum.

Staining.—Compared with the other spirochetes, the spirochete under discussion takes stain very readily with the basic anilin dyes, viz., fuchsin, methylene blue, gentian violet, etc. For the staining of smears, however, Manson's borax methylene blue, Giemsa's stain and Fontana-Tribondeau's method are especially to be recommended. For the staining of the organisms in tissues, iron-hematoxylin staining is superior to Levaditi's silver impregnation. Here Levaditi's method, although it is convenient for proving the existence of terminal flagella, is not suitable for differentiating the spirochetes in the stomach glands (Fig. 5). This is probably due to the mucus, which surrounds the organism and perhaps prevents its impregnation with silver.

As the relief staining, Benians' (1916) method is not only simple, but gives an excellent preparation (Fig. 6). The procedure is as follows: A small drop of a 2 per cent. aqueous solution of Congo red

is placed on a slide and a very small quantity of the material to be examined is rubbed into it with the platinum loop. The drop is then spread out into a rather thick film and allowed to dry. The slide is then washed over with a 1 per cent. solution of HCl in absolute alcohol and dried in the air. The film is then ready for examination.

Movement.—The movement of this spirochete is comparatively rapid and very simple. Examination under the dark-field microscope shows that the organism moves only forward and backward inflexibly in a straight line, and progression always takes place by the vibration of the posterior flagellum. Occasionally, however, there were observed a rotatory movement around the long axis, a snakelike movement, a movement forming the outline of a cone with a fixed end as apex, etc.

It must be remembered that, for examination by dark-field illumination, the material to be examined must, in most cases, be diluted with a few drops of water, otherwise the free movement of the organism is decidedly limited by compression of the tissue mass.

Distribution Among Animals.—We examined the stomachs of thirteen monkeys, forty-nine dogs, thirteen cats, twenty rabbits, fifteen guinea-pigs, thirty-eight wild rats, ten white rats, fifteen mice and fifteen field voles. All of the specimens examined were fresh, the animals having been killed only a short time before examination, except in the cases of six dead dogs and four cats, which, however, were examined shortly after death. The result of our examination is as follows:

1. The spirochete was detected in forty-three out of forty-nine dogs. Five out of six negative cases, however, were young from the same mother, only two or three weeks after weaning.

2. Out of thirteen cats eight gave a positive result, and the five negative cases were all very young.

3. Thirty-eight wild rats yielded only one positive case (*Epimys rattus alexandrinus*).

4. Thirteen monkeys were all positive.

5. Among rabbits (inoculated with *virus fixe* of rabies), guinea-pigs, white rats, mice and field voles, there was no positive case.

Judging by the foregoing results, the invasion of this spirochete seems to have a close relation to the life condition of the host. It was detected in nearly all cases of adult dogs and cats, which wander from place to place, devouring whatever food they happen to find. One hundred per cent. of the monkeys in which the spirochete is found show an extensive variation of diet. The limited life, on the other hand, may explain the rare occurrence of this organism among young dogs, young cats and wild rats, and its non-occurrence among other experimental animals, such as white rats and mice.

Distribution in the Animal Body.—We looked for the spirochete in various parts of the alimentary canals of twenty-six animals, naturally or experimentally infected; i. e., five dogs, two cats, three wild rats, five white rats, six mice and five rabbits. While the organism was always detected abundantly in the stomach of all these, in the mouth cavity and cecum, no similar spirochete could be found in any animal. But a few degenerated specimens were detected in the esophagus of four dogs, one cat, one wild rat, one white rat, one mouse and three rabbits, and still fewer in the duodenum of one dog, one white rat and one mouse. Moreover, the stomach contents were examined in four dogs, one cat, two white rats, one wild rat, one mouse and five rabbits, and only a few degenerated specimens were detected there in three dogs, one white rat and three rabbits.

These experiments indicate that the domicile of this spirochete is the stomach. Histological examination also shows that it is principally detected in the fundus gland, especially in its neck, where the organisms arranged parallel to the axis of the duct occasionally swarm so densely as to obstruct the canal. Moreover, organisms were seen lying between the chief cells or even in the cytoplasm of the parietal cells. In the case of dogs and cats, spirochetes were eventually found in the pyloric gland.

Transmission Experiment.—Mice, white rats, guinea-pigs and rabbits were selected as experimental animals. In this experiment, about five mice, two or three white rats, two or three guinea-pigs, and usually two rabbits were used for transmission from generation to generation. Here the canine strain was principally used, but occasionally the feline or the monkey strain was employed. These strains gave almost the same result.

To transplant the original strain to experimental animals of the first generation, we scraped the mucous membrane containing large quantities of this spirochete from the stomach of a dog, and fed to mice and rats in small portions and to guinea-pigs and rabbits in large amounts. After the second generation, in the case of mice and rats, a piece of the stomach wall was given to each animal of subsequent generations, and guinea-pigs and rabbits were fed a large quantity of the finely crushed mucous membrane.

Following are the results of the transmission experiment:

1. In the case of mice, we obtained the most satisfactory result, distinct multiplication being observed as early as the second day after transmission. The procedure was continued for fifteen generations, and it was found that there was a remarkable increase in every generation.

2. The transmission was also very easy in the case of white rats and the experiment was therefore discontinued after the tenth genera-

tion. The same result was obtained in the case of wild rats, where passage was continued until the fifth generation.

3. In the case of normal guinea-pigs, the transmission was very difficult. The first experiment was continued with difficulty until the third generation; by making use of animals infected by scarlet fever or measles, however, we were easily able to continue it until the tenth generation.

4. In the case of normal rabbits, we were unable to carry the procedure through the fifth generation. If the animal infected with this spirochete be inoculated with *virus fixe* of rabies, however, transmission becomes extraordinarily easy. The canine strain was thus passed without difficulty through ten generations and the feline through five generations.

Experiments on Resistance

I. LYTIC ACTION OF SAPONIN, SODIUM TAUROCHOLATE AND BILE

The material used for the experiment was the feline strain. The mucous membrane, in which large numbers of spirochetes had been detected, was scraped from the stomach of a cat immediately after death, and diluted with saline solution. Saponin and sodium taurocholate were used in 10 per cent. aqueous solution. The bile was obtained from the cat and used without being diluted.

The procedure was as follows: Equal quantities of each of these chemicals and the spirochete-containing suspension were thoroughly mixed and, at required intervals, a small drop of it was spread upon a slide by means of platinum loop. It was then dried above a weak flame (about two minutes), fixed with methyl alcohol for fifteen minutes and stained by Manson's stain under as nearly the same conditions as possible.

The result of this experiment is recorded in Table 1.

TABLE 1.—LYTIC ACTIONS OF SAPONIN, SODIUM TAUROCHOLATE AND BILE ON THE SPIROCHETE

Chemicals	15 Mins.	30 Mins.	1 Hr.	2 Hrs.	3 Hrs.
Saponin	Spirochetes became swelled and stained unfavorably. Some in process of dissolution	Staining very faint, and spirals irregular and indistinct	Almost complete dissolution	Almost complete dissolution	A very few degenerated organisms remaining
Sodium taurocholate	A few degenerated spirochetes remaining	Complete dissolution			
Bile	A few spirochetes in process of dissolution	Degenerated forms unlike the spirochete seen very rarely	Complete dissolution		
Control	No change	Degenerated forms unlike the spirochete seen very rarely	Complete dissolution	Almost complete dissolution	A very few degenerated organisms remaining

II. RESISTANCE OF THE SPIROCHETE AGAINST PUTREFACTION

(a) *Experiment in the Refrigerator.*—This experiment was made in July and August. The contents having been removed, the stomach wall containing spirochetes was placed in the refrigerator (8 to 10° C.). Every other day some of the mucous membrane was scraped off and fed to two mice, to ascertain whether it still contained live spirochetes. In three specimens from the dog and in two from the cat the results indicated that the spirochete under discussion generally continues its life in such condition for about ten days (from seven to fourteen days), showing that the death of this organism has a close relation to the putrefaction of the stomach wall. If after ten days the stomach wall putrefies to any degree and spirochetes are no longer perceived under the microscope, transmission to mice generally becomes impossible.

(b) *Experiment in a Room.*—This experiment was performed in August. The materials obtained from one dog and three mice, all heavily infected, were exposed in a room (average 30° C. in the former case and 28° C. in the latter) for twenty-four hours and the putrefied material given to two mice. Except in the case of one mouse, the result was negative. If the contents be allowed to remain in the stomach, however, this spirochete seems to disappear more quickly. We observed that the spirochete in question disappeared within about ten hours in such a stomach, even in the refrigerator. The result is probably due to the lytic action of split products of the stomach contents.

III. ACTION OF SALVARSAN ON THE SPIROCHETE IN VIVO

(a) *Infusion of Salvarsan Into the Infected Stomach.*—We selected as the experimental animals mice previously infected with large numbers of spirochetes. Arsaminol (salvarsan made in Japan) was used as an acid solution diluted only with saline solution, viz., 1 : 100, 1 : 200, 1 : 300, 1 : 500, 1 : 1,000 and 1 : 2,000. The solution was introduced directly into the stomach cavity of the mouse (1 c.c. per cap.) by means of catheter at the time of starvation. Twenty-four hours later the mice were killed and the mucous membrane of the stomachs examined under the dark-field microscope.

The result is indicated in the following table:

TABLE 2.—STERILIZATION EFFECTS OF ACID SALVARSAN SOLUTION ON THE SPIROCHETE IN VIVO

Mouse Number	Degree of Dilution of Salvarsan	Spirochete
1, 2, 3	1 : 100	—
4, 5, 6	1 : 200	—
7, 8, 9	1 : 300	—
10, 11	1 : 500	+
12, 13	1 : 1000	+
14, 15	1 : 2000	+
16, 17, 18, 19	Control	+

N.B.—Mouse 9 died about 15 minutes after infusion. Examination of the stomach revealed no spirochetes.

The table shows that the 1 : 300 acid solution of salvarsan can still sterilize the spirochete in the stomach of the mouse.

(b) *Intravenous Injection of Neosalvarsan.*—The 1 : 200 solution of neoarsaminol (neosalvarsan made in Japan) was intravenously injected into the five spirochete-bearing mice to the amount of 0.05 c.c. for 1 g. of weight, viz., in the maximum dose. Thirty hours later the mice were killed and their stomachs examined by dark-field illumination. The stomachs showed the same negative result as the controls (two normal mice).

Pathogenicity.—If the host is normal, occurrence of this spirochete in the stomach exerts no pathogenicity. If the spirochete-bearing rabbit be inoculated with the *virus fixe* of rabies, however, the spirochete abundantly increases in number and causes a specific lesion in the stomach of the host.

Such rabbits, showing rabid symptoms a week after the inoculation of the *virus fixe,* were killed, and on examination the stomach usually contained only fluid with no food particles. A large quantity of mucus always covered the surface of the mucous membrane. Marked hyperemia and hypertrophy of the mucosa, especially in the fundus, were also present. In such cases, *punctate hemorrhages, even the so-called hemorrhagic erosions, were constantly detected on both sides near the middle along the greater curvature.* Upon histological examination, the hyperemic and hemorrhagic areas were found to be located principally in the mucosa, especially in the free end of the glandular layer, but frequently also in the submucosa. The spirochetes were always abundantly detected in the lesions, where they appeared not only in the ducts of glands but sometimes even in the tissue, while they were rarely, if ever, found in the apparently normal parts. No such remarkable lesion has ever been seen in the stomach of the rabbits infected only with the *virus fixe.*

Tables 3 and 4 show the results obtained with the canine and feline strains. They also show that if, a certain interval after infection of this spirochete, the rabbit be inoculated with the *virus fixe,* the autopsy performed one week later will show that abundant increase of the spirochetes causes a severe hemorrhagic gastritis in the host. It is concluded that the infection with the *virus fixe* probably causes a gastric disturbance apparently as invisible as a very slight catarrhalic gastritis in the rabbit, and that a stomach so affected becomes a favorable medium for this spirochete. Then large increase in the number of spirochetes seems to cause the slight primary disturbance secondary to the heavy hemorrhagic gastritis. The reason for this secondary pathogenicity, however, is still uncertain. The same experiment was repeated on ten mice, and only three cases of the slight hemorrhagic gastritis were detected.

TABLE 3.—RESULT OBTAINED BY THE INOCULATION OF THE VIRUS FIXE INTO THE CANINE-STRAIN BEARING RABBITS

Rabbit No.	Generation	Date of Transmission of Spirochetes	Date of Inoculation of the Virus Fixe	Interval between Transmission and Inoculation	Occurrence of Spirochetes	Lesion of the Gastric Mucosa
1	I	May 28, 1917	May 28, 1917	0	++	—
2	I	May 28, 1917	May 28, 1917	0	—	—
3	I	May 28, 1917	May 28, 1917	0	—	—
4	I	May 28, 1917	May 29, 1917	1	—	—
5	II	June 4, 1917	June 7, 1917	3	+++	+
6	II	June 4, 1917	June 7, 1917	3	+++	++
7	III	June 14, 1917	June 18, 1917	4	+++	+++
8	III	June 14, 1917	June 18, 1917	4	+++	+++
9	IV	June 25, 1917	June 30, 1917	5	+++	+++
10	IV	June 25, 1917	June 30, 1917	5	+++	++
11	V	July 7, 1917	July 9, 1917	2	++	—
12	V	July 7, 1917	....	...	—	—
13	VI	July 16, 1917	July 22, 1917	6	?	+++

TABLE 4.—RESULT OBTAINED BY THE INOCULATION OF THE VIRUS FIXE INTO THE FELINE-STRAIN BEARING RABBITS

Rabbit No.	Generation	Date of Transmission of Spirochetes	Date of Inoculation of the Virus Fixe	Interval between Transmission and Inoculation	Occurrence of Spirochetes	Lesion of the Gastric Mucosa
14	I	July 9, 1917	....	...	—	—
15	II	July 19, 1917	July 23, 1917	4	+++	+++
16	III	July 30, 1917	Aug. 7, 1917	8	+++	+++
17	IV	Aug. 14, 1917	Aug. 20, 1917	6	+++	+++
18	IV	Aug. 14, 1917	Aug. 20, 1917	6	+++	+++
19	V	Aug. 27, 1917	Aug. 29, 1917	2	+++	++
20	V	Aug. 27, 1917	Aug. 31, 1917	4	+++	++
21	V	Aug. 27, 1917	Sept. 1, 1917	5	+++	+++
22	V	Aug. 27, 1917	Sept. 3, 1917	7	+++	+++
23	VI	Sept. 5, 1917	Sept. 6, 1917	1	+++	+
24	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++
25	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++
26	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++

N. B.—(1) Under "Occurrence of spirochetes": — indicates negative result; + from one to several spirochetes in a preparation; + + from one to several in several fields; + + + several or more in a field.

(2) Under "Lesion of the gastric mucosa": — indicates the apparent absence of lesions; + hyperemia and hypertrophy moderate, hemorrhage very slight, gastric contents juicy and mucus abundant; + + hyperemia and hypertrophy distinct, hemorrhage moderate and contents fluid, with only a small quantity of floating solid particles; + + + hemorrhage remarkable and contents completely fluid.

(3). Rabbits 12 and 14 are controls, which were only subjected to the inoculations of the *virus fixe*.

(4). Rabbit 13 died in the early morning on the day on which we intended to kill it; its stomach with the contents was immediately placed in the refrigerator. About ten hours later, upon examination of the stomach, no spirochetes could be detected, while marked hemorrhage was observed. This shows that, in all probability, the spirochetes were dissolved by the split products of the stomach contents.

Moreover, the stomachs of guinea-pigs previously infected with measles or scarlet fever and fed with the spirochetes constantly showed a great increase of the spirochete and the distinct hyperemia and hemorrhage of the mucosa.

The conclusion may be drawn from these results that the cases of hemorrhagic gastro-enteritis described by Balfour and Lucet are in all probability due to the secondary pathogenicity of this spirochete.

As a sequel to the foregoing experiment, we inoculated the emulsion of the gastric mucosa, containing large numbers of this spirochete, into the testes of four white rats, but no multiplication of the organism was found to have occurred.

SUMMARY

1. The stomach spirochete is an inflexible spiral with a straight longitudinal axis, the transverse section being an ellipse. It is provided with a flagellum at each end.

2. It takes stain very readily, compared with the other spirochetes, not only by the basic anilin dyes commonly used for the staining of bacteria, but by iron hematoxylin.

3. Its movement is comparatively active and very simple, progression being only forward and backward in a straight line.

4. This organism was detected in forty-three out of forty-nine dogs, in eight out of thirteen cats, in one out of thirty-eight wild rats and in every one of thirteen monkeys, but was absent in twenty rabbits, fifteen guinea-pigs, ten white rats, fifteen mice and fifteen field voles.

5. Its domicile is the stomach, especially the fundus gland.

6. It is readily soluble in saponin, sodium taurocholate and bile. It is also labile to putrefaction.

7. The introduction of salvarsan into the stomach is easily capable of sterilizing the spirochetes domiciling there.

8. The organism is readily transmitted to the stomach of the rat or mouse, but transmission to the normal rabbit or guinea-pig is very difficult.

9. If, after a certain interval, a rabbit previously infected with the spirochete be again inoculated with the *virus fixe,* the stomach of the host, at autopsy performed a week after inoculation, shows a distinct increase of spirochetes and a remarkable hemorrhagic inflammation in the mucosa. The same result was obtained in guinea-pigs previously infected with scarlet fever or measles after subsequent feeding with this spirochete.

We wish to express here our deep indebtedness to Prof. S. Kitasato, director of the Kitasato Institute, and to Profs. S. Hata and S. Kusama for their cordial guidance.

References Cited

Balfour, A. 1906.—A haemogregarine of mammals and some notes on trypanosomiasis in the Anglo-Egyptian Sudan. Jour. Trop. Med., 9:81-92.

1906a.—Trypanosomiasis in the Anglo-Egyptian Sudan. II. Rep. of Wellcome Res. Lab. at Gordon Mem. Col. Khartoum, pp. 113-173.

Ball, V. et Roquet, M. 1911.—Spirochètes et affections hémorrhagiques gastro-intestinales du chien. Jour. Med. Vét. et Zoot., 14:257-260.

Benians, T. H. C. 1916.—Relief Staining for Bacteria and Spirochaetes. Brit. Med. Jour., 2:722.

Bizzozero, G. 1893.—Sulla presenza di batteri nelle ghiandole rettali, e nelle ghiandole gastriche del cane. Atti R. Accad. Sci. Torino., 28:249-251.

Carnot, P. et Lelièvre, A. 1909.—Sur la double ordination des cellules bordantes de l'estomac. Compt. rend. Soc. Biol., 66:147-149.

1909a.—Morphologie du produit d'excrétion des cellules bordantes. Compt. rend. Soc. Biol., 66:311-313.

Kasai, K., and Kobayashi, R. 1917.—Description of Various Species of the Spirochete Found in the Alimentary Canal of Wild Rats and Guinea-Pigs. Saikingaku Zasshi, No. 259.

Krienitz, W. 1906.—Ueber das Auftreten von Spirochaeten verschiedener Form im Mageninhalt bei Carcinoma ventriculi. Deut. med. Wchnschr., 32:872.

1906a.—Ueber morphologische Veränderungen an Spirochaeten. Centralbl. Bakt., I, Orig., 42:43-47.

Lucet, M. 1910.—Sur la présence de spirochètes dans un cas de gastro-entérite hémorrhagique chez le chien. Compt. rend. Acad. Sci., 151:260-262.

1909a.—Sur les spirilles parasites des glandes gastriques du chien et du chat. Compt. rend. Soc. Biol., 66:617-618.

Salomon, H. 1896.—Ueber das Spirillum des Säugetiermagens und sein Verhalten zu den Belegzellen. Centralbl. Bakt., I, Orig., 19:433-442.

Suda, G. 1916.—On the Method of Staining Gland Cells in the Gastric Gland. Kyoto Igaku Zasshi., 3:273.

Regaud, C. 1909.—Sur une curieuse localisation de spirilles parasites dans les canalisations glandulaires de la muqueuse gastrique normale, chez le chien et le chat. Compt. rend. Soc. Biol., 66:229-231.

Description of Plate

Fig. 1-3.—Three types of the spirochete.

Fig. 4.—Various forms of the spirochete under the dark-field microscope.

Fig. 5.—Various forms of the spirochete stained with Levaditi's method.

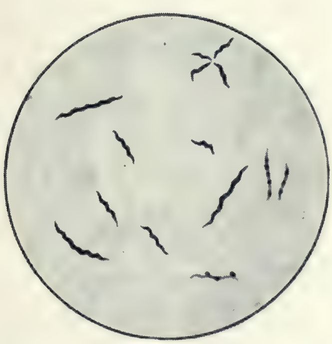
Fig. 6.—Figures of the spirochete treated with Benians' relief staining.

1

4

2

5



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6

PLATE I

ON THE SPECIFIC IDENTITY OF *HERONIMUS CHELYDRAE* MacCALLUM AND *AORCHIS EXTENSUS* BARKER AND PARSONS

Horace W. Stunkard

The monostomes are among the least known of North American trematode groups. Records give descriptions of only six species, each the single representative of a genus, and according to the classification of Ward (1918) belonging to four different families. Existing descriptions in most cases are far from complete and data necessary for taxonomic determination are lacking. This deficiency has been pointed out by other workers, both in this country and in Europe, and the classification of the monostomes is not well established. In fact, certain investigators regard them as aberrant forms, sprung from different distome groups, which alike have lost the acetabulum. If this is true and the similarity is merely superficial, the present system of classification must be entirely revised. Careful, complete descriptions of these forms are necessary to provide the data for a natural system of classification. The present study, it is hoped, will prove a step toward the solution of this problem.

Heronimus chelydrae was described by MacCallum (1902) from the lungs of the American snapping turtle, *Chelydra serpentina,* taken at Ontario, Canada. After the description of the form, the author stated, "It seems necessary, therefore, to establish a new genus in the family Monostomidae to accommodate this form—a genus which stands far apart from the other genera in several respects, but especially in the position and nature of the genital opening, in the complicated structure and course of the uterine tract, the unusual formation of the yolk glands, in the presence of but one testicle, and in the position of the excretory pore."

Barker and Parsons (1914) in a preliminary announcement described a monostome, parasitic in the lungs of *Chrysemys marginata,* which they named *Aorchis extensus.* In a later paper (Barker and Parsons, 1917) they gave a more extended description of the form based on the study of the specimens originally secured from Lake Emily, Minnesota, and others found later in the lungs of the same host taken from the Mississippi River near Fairport, Iowa. As diagnostic characters of the genus Aorchis they stated:

"Body medium to large, slightly tapering toward the anterior and posterior ends, posterior end rounded. Oral sucker small, weak but distinct. Mouth opening terminal. Pharynx strongly muscular, with-

out pockets. Esophagus short. Intestine composed of two simple blind sacs, not uniting at the posterior end. Genital pore not prominent, ventral to the pharynx, close to oral sucker. Ovary anterior between intestinal ceca. Shell gland compact, posterior to and smaller than ovary. Uterus made up of coils which fill the body lateral to and overlap the intestinal ceca, extending from level of the ovary to posterior end of the body and two straight and parallel uterine tubes which pass anteriorly up the median axis of the body between the intestinal ceca. Vitelline gland a voluminous, coarse, compact, U-shaped mass posterior to ovary and dorsal to the intestine, with the closed portion at the anterior end. Protandrous. Testis absent, or atrophied in old worms. A single ovoidal testis present in young worms, anterior, caudad to ovary. Prostate gland near testis, seminal vesicle, a large tubular structure extending from genital pore to the level of the second anterior fifth of the body. Protrusible nonmuscular cirrus present. Laurer's canal and seminal receptacle absent. Eggs without lids. Excretory pore posterior dorsal."

A new family, Heronimidae, was created by Ward (1917) to contain the two genera Heronimus and Aorchis. This author had collected specimens which he stated probably belonged to the species *A. extensus,* from the lungs of various turtles from Michigan, Indiana, Illinois and Nebraska. Comparing the two genera he says, "These two forms are so much alike that they may prove to be identical, or at least to belong to the same genus, but they are in some respects very different from any other monostomes known, and I have established for them a new family with the following characters:

"Heronimidae Ward. Moderate sized monostomes with thick, elongate, soft body, slightly flattened, tapering toward both ends. Oral sucker weak, pharynx large, esophagus short or absent; ceca simple, narrow, extending to posterior tip but not united. Vitellaria compact tubular; uterus with four longitudinal regions; genital pore ventral to oral sucker, near anterior tip. Testis tubular, small, copulatory apparatus poorly developed. In lungs of turtles, northern North America."

The following year, Ward (1918) restated the family characters with the following additions: "Vitellaria compact tubular, shaped like an inverted V. Testes tubular, lobed or with short branches, united into a V-shaped organ with the apex anteriad. . . . Two genera imperfectly known which may prove to belong in the same genus." The genera Heronimus and Aorchis he distinguished as follows:

"Vitellaria extend only half way from ovary to posterior end. Seminal receptacle present . . . Heronimus MacCallum 1902.

"Vitellaria extend from ovary to posterior end of body. Seminal receptacle absent. . . . Aorchis Barker and Parsons 1914."

Ward (1918) gave two figures of *A. extensus* and a brief specific description. He differed from Barker and Parsons with regard to the testis. Ward found "Testes elongate, tubular, irregularly lobed."

While engaged for several years in the study of animal parasites, I have examined about three hundred turtles and found a large monostome in the lungs of six different species collected from the central and southern as well as northern districts of North America. This parasite has been secured from *Chelydra serpentina* taken in Iowa, Illinois, Ohio, North Carolina and Texas. Specimens have been found in the lungs of *Chrysemys marginata* taken in Iowa, Illinois, Missouri and Kentucky; *Pseudemys elegans* and *Malacoclemmys geographicus* in Illinois; *Aromochelys odoratus* and *Kinosternum pennsylvanicum* in North Carolina.

This form I had regarded as identical with *A. extensus* Barker and Parsons and the close similarity to *H. chelydrae* had been noted. Through the kindness of the director of the U. S. National Museum, I have had an opportunity recently to study the specimens of *Heronimus chelydrae* deposited there by MacCallum. In addition, a large specimen of *H. chelydrae* from the collection of Albert Hassall has been placed at my disposal. This worm is from the lung of *Kinosternum pennsylvanicum* taken near Baltimore, Maryland. I wish to express here my thanks to these workers for their kind assistance.

A careful examination of the specimens and comparison with the description of MacCallum confirms his observations. He gave a careful description of the morphology of the parasite and a detailed histological description of certain structures. He described the uterus as extensively convoluted and folded, traversing the length of the body four times, but he did not describe the definite course of the tube and his figure gives a diagrammatic representation rather than a precise picture of the position of the loops and coils of the uterus. Further, he did not describe the extent of the testis or state that in certain specimens this organ is reduced or degenerate.

Comparison of the monostomes I have collected with the type specimen and other sectioned individuals of *H. chelydrae* shows fundamental and precise agreement in every respect and demonstrates that they belong to that species.

A careful study of the material of *H. chelydrae* yields results strikingly different from those of Barker and Parsons. The difference in certain features is so marked that I may be dealing with another species, but in other respects there is such precise agreement that I am inclined to believe I have the same form. Some of the specimens at hand are from the same host and the same locality where Barker and Parsons' material was collected. They agree with the description of Barker and Parsons in size and shape, size and character of the oral

sucker, pharynx, esophagus and digestive ceca, extent and position of the uterine coils, size and position of the ovary, character of the copulatory organs, and location of the genital pore. But in *H. chelydrae* the excretory system is different from that described by Barker and Parsons, the pore is near the anterior instead of the posterior end of the body, the vitellaria are ventral and not dorsal, a seminal receptacle is present, also a V-shaped testis which corresponds in size and extent with Barker and Parsons' description of the vitellaria. If these differences were minor in character, I should conclude that the specimens belong to a different species, but the form of the excretory system is a fundamental and characteristic feature of large groups, and questions that involve the dorso-ventral axis or concern the form of the testis and vitellaria are not of specific nature. Consequently, in view of the agreement in other respects, I am inclined to question the accuracy of Barker and Parsons' description. In their first report (Barker and Parsons, 1914) the eggs are described as possessing a short polar stalk and a statement is made that the cirrus is lacking. Their later paper (1917) does not refer to any polar stalk on the eggs and a cirrus is described. Ward (1918) has made further corrections to the description. The comparison of *H. chelydrae* with the description of *Aorchis extensus* leaves little if any doubt that the two forms are identical. Barker and Parsons do not refer to the work of MacCallum and have not published a comparison of their form with *H. chelydrae*. The unsatisfactory nature of Barker and Parsons' description and the agreement of the parasites described by them with *Heronimus chelydrae* discredits the validity of the genus Aorchis and the name should be suppressed.

Supplementing the work of MacCallum, I wish to make certain additions to the description of *Heronimus chelydrae*. In the examination of turtles, the heaviest infection found was six flukes in one host, and though the parasite is not uncommon, the relative infection was slight. On the findings in three male and two female turtles, Barker and Parsons endeavor to show the females more heavily infected than the males. Such a conclusion seems unfounded, and in the examination of over fifty infected turtles, I find practically no difference as far as sex of host is concerned.

The body of the living worm is usually curved and often assumes the form of a double bend like an elongated S, the short anterior region becomes concave ventrally and the long posterior part concave dorsally. Movement is slow and sluggish. The size and shape of the parasite have been described by both MacCallum and Barker and Parsons. Apparently the measurements of both were made from fixed specimens, and this can account for the slight difference in their reports. I have observed specimens that measured 18 mm. when fully extended that

did not exceed 12 mm. in the normal characteristic form. The relative width naturally varies with the amount of elongation. MacCallum described the cuticula as sometimes thrown into slight folds or rugae independent of the musculature. Barker and Parsons described strong circular bands of muscle fibers which run around the body at regular intervals and give it a segmented appearance when contracted. I have observed the same conditions, the slight folding of the cuticular covering and also the more pronounced constrictions involving the muscular wall. But in sections I have been unable to demonstrate any regularly occurring bands of strong circular muscle fibers or even an intermittent thickening of the circular muscles of the body wall. The musculature is very weak and slight, no one of the three layers which form the body wall is strongly developed. Lying inside the body wall there is a compact layer of parenchyma (Figs. 3, 5, 6, 9), and this layer increases in thickness anterior to the ovary. Within the outer thickened stratum the parenchyma has the loose vacuolated appearance (Figs. 3, 9) well described by MacCallum.

The measurements of the oral sucker, pharynx, esophagus and intestinal ceca agree with those given by Barker and Parsons, and my examination confirms the histological description of these structures as given by MacCallum.

MacCallum described the ovary as located on the left side of the body. Barker and Parsons' statement concerning the size and position of the ovary agrees with that of MacCallum except that they found the ovary on either the right or left side. My observations confirm the statement of Barker and Parsons; the ovary may be on either the left or right side, but is always on the side opposite from the anterior pigmented region of the uterus. The description of the structures which comprise the oötype as given by MacCallum is entirely confirmed by my observation. A seminal receptacle is present (Fig. 3). It is about one fourth the size of the ovary, situated on the postero-median side of the ovary. It agrees in size and position with Barker and Parsons' description of the testis. In the specimens in which I have been able to trace the course of the uterus, it passes from the oötype to the side of the body opposite from the ovary and posteriad in many coils around the intestine and testis to the posterior end of the body. Then it bends forward and continues anteriad on the ovarian side in similar coils and loops to the level of the ovary, where it turns posteriad and passes diagonally to the opposite side of the body. Here it turns anteriad and soon becomes heavily pigmented. It extends in many loops and folds to or slightly farther than the level of the ovary and then turns caudad, extending as a straight tube in the dorso-median line almost to the posterior end of the body. The pigmentation diminishes as the tube passes caudad and in many specimens disappears about

midway between the ovary and the posterior end of the body. At its posterior end, the descending median section of the uterus turns ventrad and opens into the large, median, ventral sac-like portion (Fig. 7) which extends cephalad to the metraterm. The histological character of the various regions of the uterus has been described by MacCallum and the character of the copulatory organs and position of the genital pore by both MacCallum and Barker and Parsons.

The vitellaria (Figs. 7, 10, 11) consist of two glandular structures which meet anteriorly to form the vitelline receptacle and extend almost to the posterior end of the body. They lie median and ventral to the ceca, the anterior part is tubular, but the central and posterior regions are solid and rodlike.

The eggs are very thin-shelled and, when massed together in the uterus, lose their characteristic shape and become many-sided. Often the shells are lost entirely, the embryos develop eye spots, and in the large sac-like terminal portion of the uterus, there are fully developed ciliated miracidia. This species offers, then, an opportunity to follow the development of the embryo from the maturation and fertilization of the egg to the first larval stage.

The testis (Figs. 7, 8, 10, 11) is a large U- or V-shaped structure; the closed portion is cephalad and situated one fourth to one fifth of the body length from the anterior end. The crura extend caudad to a level about one eighth of the body length from the posterior end. As described by Ward, they are elongate, tubular, irregularly lobed. Their histological appearance is well described by MacCallum and is shown in Figs. 7, 8, 11. Barker and Parsons described a condition in which the testis is atrophied and degenerate, and stated that this is true particularly in the "older larger worms." I have observed the same condition in many individuals of *H. chelydrae*. Often, however, the organ is degenerate and the crura of the testis have shrunk to mere strands of tissue, and sections show the cells in a state of degeneration and disintegration. On the other hand, however, in many of the largest individuals the testis is full-sized and sections show vigorous functional activity of the cells. I am at a loss to account for the atrophy of the testis, but since it occurs in a large percentage of small individuals, and does not occur in many of the largest, I am not inclined to regard the conclusion of Barker and Parsons as satisfactory.

A short vas deferens arises from the anterior part of the testis, turns ventrad and caudad where it opens into the posterior end of the seminal vesicle. No prostate gland was found. The seminal vesicle extends caudad only a short distance from the median part of the testis and the posterior part of the vesicle is usually irregularly coiled. The vesicle extends anteriad in the ventral part of the body and just anterior to the ovary passes over into the cirrus sac (Fig. 4). This sac is

approximately the same width as the vesicle and the wall is not strongly muscular. The copulatory structures have been discussed.

The excretory system consists of a large, median, dorsal collecting vesicle and smaller ducts distributed throughout the tissue of the body. The large dorsal collecting vesicle extends from the pharyngeal region almost to the posterior end of the body and opens to the exterior in the median dorsal line just posterior to the pharynx. Its walls are often folded and it lies in loose vacuolated parenchymatous tissue. No definite branches from this vesicle were demonstrated. The two largest excretory ducts (Fig. 6) arise in the region of the oral sucker and pass caudad, one on either side of the body, ventral and median to the ceca. They become smaller and more dorsal in position as they extend posteriad and near the posterior end of the seminal vesicle they disappear in the loose parenchyma.

SUMMARY

The present study demonstrates the specific identity of *Heronimus chelydrae* MacCallum and *Aorchis extensus* Barker and Parsons. It confirms the work of MacCallum and includes many additions to the description of the species. The course of the uterus is traced and the position, extent and atrophy of the testis is demonstrated. The wide distribution of the species, and its infestation of six different species of turtles, are items of interest and importance.

BIBLIOGRAPHY

Barker, F. D., and Parsons, S. 1914.—A New Species of Monostome from the Painted Terrapin, *Chrysemys marginata*. Zool. Anz., 45: 193-194.

1917.—A Monostome Lung Fluke from the Painted Terrapin, *Chrysemys marginata* Agassiz. Trans. Amer. Micr. Soc., 36: 55-66, 2 pl.

MacCallum, W. G. 1902.—*Heronimus chelydrae,* nov. gen., nov. sp. A New Monostome Parasite of the American Snapping Turtle. Centr. Bakt. Par., Orig., 32: 632-636, 2 figs.

Ward, Henry B. 1917.—On the Structure and Classification of North American Parasitic Worms. Jour. Paras., 4: 1-12.

1918.—Parasitic Flatworms, Trematoda. Fresh-Water Biology. Ward and Whipple.

Explanation of Figures

(All camera lucida tracings)

1. Type specimen of *Heronimus chelydrae,* dorsal view, × 8.

2. Ovary and oötype region, from large mounted specimen, × 25.

3. Sagittal section showing ovary, seminal receptacle, vitelline receptacle and beginning of uterus, × 57.

4. Sagittal section showing excretory pore, genital pore, extent of cirrus sac, and relation of pharynx and esophagus, × 57.

5. Cross section at posterior end of pharynx, showing nerve commissure and relation of uterus and cirrus sac, × 57.

6. Cross section at level of excretory pore, showing uterus, cirrus sac and small anterior excretory ducts, × 57.

7. Cross section near the posterior end of the body, just anterior to the point where the median descending section of the uterus opens into the large ventral sac-like portion. The vitellaria and crura of the testis extend posterior to this level and appear in the section, × 57.

8. Sagittal section, lateral to intestine, showing testis and uterine coils, × 27.

9. Cross section thru body at anterior tip of ovary, × 48.

10. Cross section at the level of the oötype, showing anterior tip testis, tubular nature of the anterior part of the vitelline gland, large sac-like nature of the terminal portion of the uterus and the smaller pigmented descending section of the uterus dorsal to it, × 42.

11. Cross section just anterior to the bifurcation of the testis, showing the rod-like character of the vitellaria, × 29.

12. Sagittal section showing oral sucker and pharynx, genital pore, uterus and cirrus sac, × 87.

Abbreviations Used in Figures

cs — cirrus sac
ed — excretory duct
ev — excretory vesicle
ep — excretory pore
i — intestine
mg — Mehlis' gland
nc — nerve commissure
o — ovary
od — oviduct
os — oral sucker
ph — pharynx
sv — seminal vesicle
sr — seminal receptacle
t — testis
u — uterus
v — vitellaria
vr — vitelline receptacle

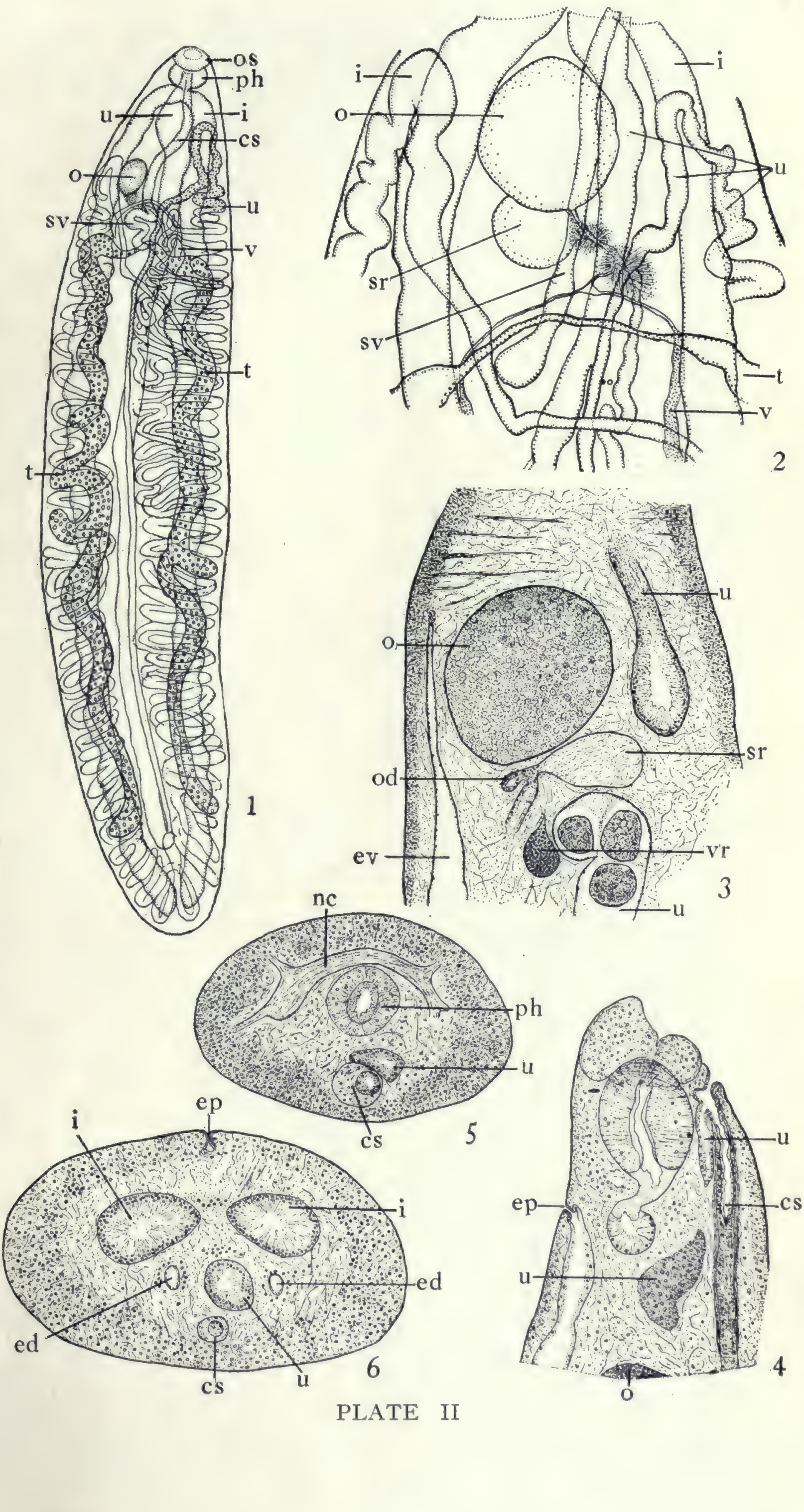
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PLATE II

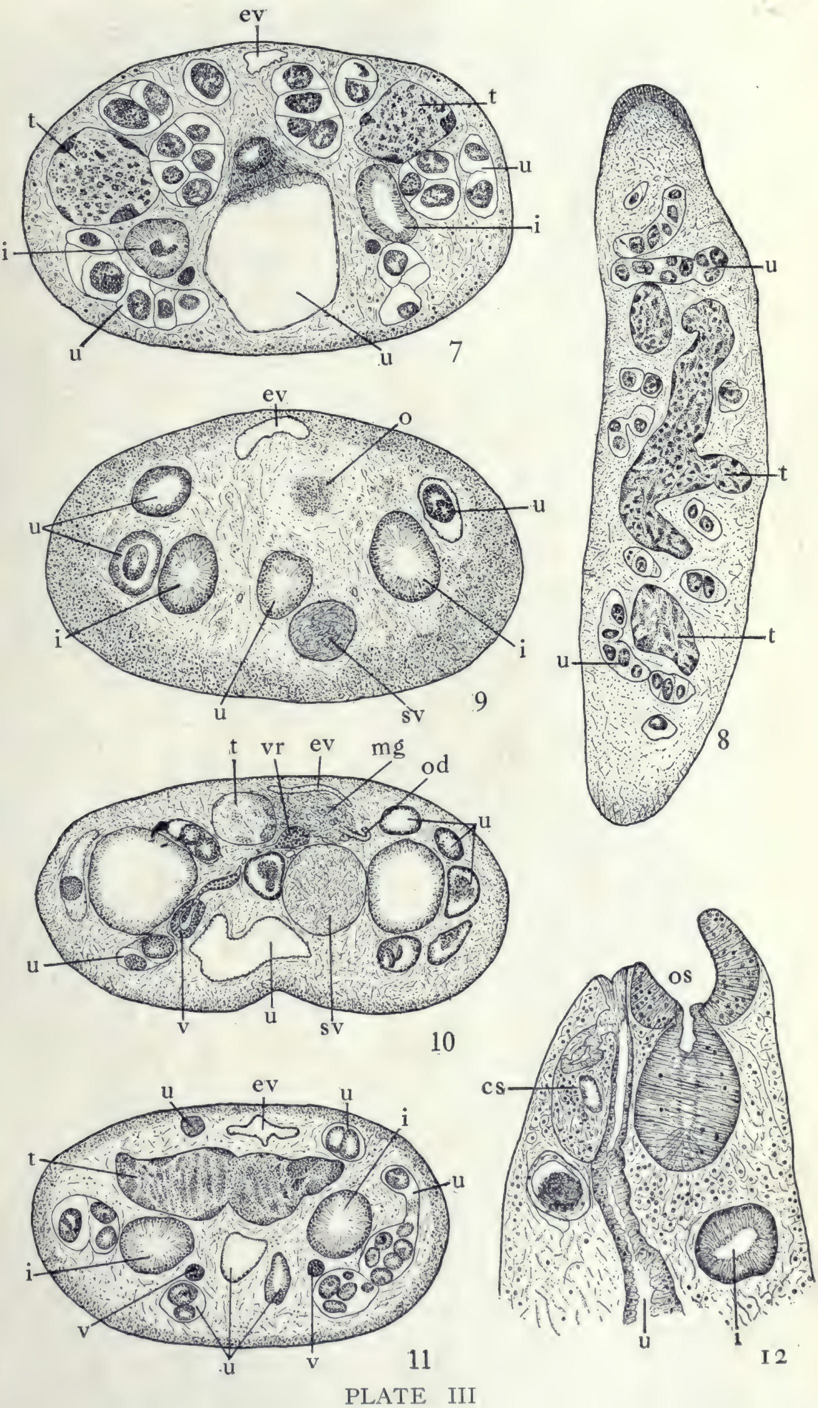
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PLATE III

ON THE MIGRATING COURSE OF ASCARID LARVAE IN THE BODY OF THE HOST

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In my previous paper (Yoshida, 1919) I have reported that the larvae hatched out in the intestine of a feeding animal, migrate into the liver, lungs and trachea successively and they again pass down the alimentary canal to the intestine, being finally evacuated with the feces sooner or later. The exact course of migration, however, remained to be reported. Since April of this year I have devoted myself to decide experimentally the general course of migration by the larvae in the body of the host. For this purpose, two sets of experiments have been made, (1) experiments injecting larvae into the body of the animal and (2) feeding experiments with the ripe eggs. In the first set of experiments, the migratory power of the larvae was examined chiefly and in the second set the natural and actual course of migration was observed. Both sets of experiments gave successful results from which I have learned the general course of migration from the intestine to the lung.

Before proceeding to describe the experiments, it seems necessary to mention here briefly my methods for injecting larvae and finding them in various organs and tissues. The larvae in the pleural or abdominal cavity are easily collected by centrifuging the fluid in the cavity. As washing fluid 10 or 20 c.c. of physiological salt solution was usually used.

For collecting larvae in any organ, the tissue, such as liver, lungs, etc., is gently brayed in the earthenware mortar with a small quantity of physiological salt solution, the particles of the organ are mixed with 10 or 20 c.c. of physiological salt solution and then filtered through a wire net or gauze of the definite size of mesh to remove the coarse particles. The larvae finally are easily collected by centrifuging the filtered fluid. The coarse particles are to be repeatedly brayed and filtered as before until larvae are no longer present. The larvae thus collected from any organ or tissue are injected with 2 or 5 c.c. of physiological salt solution. Injection was made into the subcutaneous layer, abdominal or pleural cavity of guinea-pig.

Injection Experiments

As to the migrating power of the ascarid larvae, results of two injecting experiments were reported in my previous paper. Since then

numerous experiments were tried by injecting the larvae into the abdominal or pleural cavity, or into the subcutaneous layer of the animal.

Exp. 1.—At 1 p. m., on June 6, larvae collected from the liver of a guinea-pig, killed seventy-five hours after feeding with ripe eggs, were injected into the abdominal cavity of a guinea-pig A. On the next morning after, about twenty hours, the animal was found dead. In the pleural cavity were found two living larvae, but none in the lungs. In the abdominal cavity numerous dead larvae were present and a few in the liver.

Exp. 2.—At 1 p. m., on June 6, larvae from the liver of the same animal as in the preceding experiment were injected into the pleural cavity of two guinea-pigs, C and D. On the next morning the animal C was found dead after twenty hours; in the pleural cavity numerous dead larvae were present and a few living ones in the lungs.

Exp. 3.—The animal D was also found dead in the same morning, the result of examination was the same as in the preceding case.

Exp. 4.—At 8 a. m., on June 4, larvae from the lungs of a guinea-pig, killed sixty-five hours after feeding, were injected into the abdominal cavity of a guinea-pig B. On the next morning the animal was found dead after twenty-four hours. The lungs and liver were infected, many dead larvae being in the abdominal cavity and a few living in the pleural.

Exp. 5.—At 9 a. m., on June 10, larvae from the liver of an animal, killed sixty-six hours after feeding, were injected into the abdominal cavity of a guinea-pig B. The animal was found dead on the next morning after twenty-four hours. Numerous dead and living larvae were found in the abdominal cavity, a few in the liver, five in a piece of the lung and none in the pleural cavity.

Exp. 6.—At 2 p. m., on June 10, the larvae from the liver of the guinea-pig, killed seventy-one hours after feeding, were injected into the abdominal cavity of two guinea-pigs C and D. In the evening of the next day, the animal C was found dead after about twenty-seven hours. On dissection it was discovered that large quantities of the injected matter were accumulated between the skin and the underlying muscle layer. (It was generally observed that the injected particles of the lung or liver with the larvae were gathered into masses in the abdominal or pleural cavity into which they were injected.) In the masses of the injected particles of the liver, larvae were present and dead larvae were found in the abdominal cavity, a few living ones in the liver; they were relatively numerous in the lungs while none were in the pleural cavity.

Exp. 7.—Animal D of the preceding experiment died in the same evening; the result of examination was quite the same as C.

Exp. 8.—A 8 a. m., on June 3, larvae from the liver of a guinea-pig, killed forty-eight hours after feeding, were injected into the abdominal cavity of two guinea-pigs C and D. Animal C was killed at 8 a. m. on the 5th, after forty-one hours. An abundance of larvae were present in the lungs and a few in the liver, while none were found in the pleural and abdominal cavity.

Exp. 9.—Animal D was killed at 3 p. m. on the 5th, after forty-three hours. Numerous larvae were found in the lungs, but none in the pleural cavity and a few in the liver.

Exp. 10.—At 9 a. m., on June 10, larvae from the lungs of a guinea-pig, killed sixty-six hours after feeding, were injected into the abdominal cavity of guinea-pig A. The animal was killed at 4 p. m. on the 12th, after fifty-five hours. A few larvae were present in the lungs and the other organs were unexamined.

Exp. 11.—At 2 p. m., on June 4, larvae from the liver of a guinea-pig, killed after seventy-one hours after feeding, were injected into the abdominal cavity. The animal was killed at 8 a. m. on the 7th, after sixty-six hours. I found two specimens of larvae in the pleural cavity, four in the abdominal cavity, a few in the lungs and a very few in the liver.

Exp. 12.—At 2 p. m., on the 4th, larvae from the lungs of the same guinea-pig were injected into the abdominal cavity of animal B. It was killed at 10 a. m. on the 10th, after ninety-two hours. On the dissection it was found that the injected materials were all introduced into between the skin and the underlying muscle layer. In the accumulated masses of the injected materials larvae were present and a few larvae in the liver as well as in the abdominal cavity. None in the lungs and pleural cavity.

From the observation in this experiment we may easily believe that the larvae migrate into the body cavity through the muscular wall of abdomen.

Exp. 13.—At 11 a. m., on May 10, larvae from the lungs of the guinea-pig, killed after ninety-one hours from feeding, were injected into the abdominal cavity. The animal was killed at 8 a. m., on the 15th, after 117 hours. A few larvae were found in the lungs, but none in the liver.

Exp. 14.—At 11 a. m., on May 11, larvae from the lungs of the guinea-pig, killed after ninety-one hours from feeding, were injected into the abdominal cavity. It was killed at 9 a. m. on the 16th, after 118 hours. The lungs were tolerably hemorrhaged and the larvae were present.

Exp. 15.—At 10 a. m., on May 4, the larvae from the liver of a guinea-pig killed after sixty-nine hours from feeding, were smeared over the abdominal skin from which the hair was removed by cutting closely and shaving. The animal was fixed on the holder during about four hours after operation, restricting the violent movement in order to prevent the loss of larvae and to avoid larvae being taken into the mouth of the animal. After 5 p. m. the animal was put in the separate cage and the smeared part of abdomen was closely covered by the cloth. In the next morning the animal was put in the same cage as others. At noon on the 11th larvae from the liver of a guinea-pig killed after 118 hours, were smeared on the nape of the neck. The animal was killed at 9 a. m. on the 17th, after thirteen and six days from the first and the second smearing, respectively. Five specimens of larvae were obtained from a piece of the lung, two in a part of the large intestine and none in the liver. Almost all these larvae were fully developed, being about 1.8 mm. long. This experiment shows evidently that larvae on the skin may pierce through the body wall of host. It also proves how important and necessary the lungs are for the further development of the ascarid larvae.

From observations in above experiments it may be easily recognized that the larvae injected into the pleural cavity penetrate into the lungs directly, as Experiments 2 and 3 show, and those injected into the abdominal cavity penetrate into the liver or pierce the diaphragm to enter the pleural cavity and thence to reach the lungs. These facts show great power of the larvae in boring through various tissues, as skin, muscle, tendon and several parenchymatous tissues.

Some larvae in the abdominal cavity might have reached the lungs passing through the liver and heart by the way of a blood vessel, but the majority of them are considered to have proceeded directly to the lungs piercing through the diaphragm. It is reasonable to think so from the nature of the larvae, which have marked power in boring

TABLE 1

No.	Date Injected	Injecting Place	Age of Larvae	Date Killed	Duration of Infestation	Pleural Cavity	Lungs	Abdominal Cavity	Liver
1	1 p. m. June 6 A	Abdominal cavity	75 hours from liver	Died morning June 7	< 20 hr.	3	None	Many dead larvae	Present
2	1 p. m. June 6 C	Pleural cavity	75 hours from liver	Died morning June 7	< 20 hr.	Present	A few		
3	1 p. m. June 6 D	Pleural cavity	75 hours from liver	Died morning June 7	< 20 hr.	Present	A few		
4	8 a. m. June 4 B	Abdominal cavity	65 hours from lung	Died morning June 5	< 24 hr.	A few	Present	Dead larvae	Present
5	9 a. m. June 10 B	Abdominal cavity	66 hours from liver	Died morning June 11	< 24 hr.	None	5	Present	A few (? in a piece)
6	2 p. m. June 10 C	Abdominal cavity	71 hours from liver	Died evening June 11	< 27 hr.	None	Many	Many	A few
7	2 p. m. June 10 D	Abdominal cavity	71 hours from liver	Died evening June 11	< 27 hr.	None	Many	Many	A few
8	3 p. m. June 3 C	Abdominal cavity	48 hours from liver	Killed 8 a. m. June 5	41 hr.	A few	Many	None	A few
9	3 p. m. June 3 D	Abdominal cavity	48 hours from liver	Killed 3 p. m. June 5	48 hr.	None	Many	A few	A few
10	9 a. m. June 10 A	Abdominal cavity	66 hours from liver	Killed 4 p. m. June 12	55 hr.	None	A few	None	None
11	2 p. m. June 4 C	Abdominal cavity	71 hours from liver	Killed 8 a. m. June 7	66 hr.	2	A few	4	A few
12	2 p. m. June 4 D	Subcutaneous	71 hours from lung	Killed 10 a. m. June 8	92 hr.	None	None	A few	A few
13	11 a. m. May 10	Abdominal cavity	91 hours from lung	Killed 8 a. m. May 15	117 hr.	......	A few	......	None
14	11 a. m. May 11	Abdominal cavity	91 hours from lung	Killed 9 a. m. May 16	118 hr.	......	Present		
15	1 p. m. May 4, noon May 11	Smeared on abdominal and nape	69 hours and 91 hours	Killed 9 a. m. May 17	13 or 6 days	......	5 and 2 in large intestine		

tissue. Moreover, the appearance of larvae in the pleural cavity clearly proves their direct migration from the abdominal cavity.

FEEDING EXPERIMENTS

Such power of larvae in boring through the tissues makes it reasonable to think that larvae hatched in the intestine may pierce through the intestinal wall to enter the abdominal cavity. This is also surely and actually confirmed by the following feeding experiments.

Feeding experiments were chiefly intended to examine whether the larvae just escaped from the eggshell in the intestine of host may pierce through the intestinal wall or not, and to trace the subsequent course of migration made by the larvae.

Exp. 16.—At noon on June 14, two guinea-pigs A and B were fed with the ripe eggs of sixty-one days old, and the A was killed at 8 a. m. on the next day after twenty hours. The lungs were slightly hemorrhaged, spotted with bloody color here and there, contained numerous larvae of Ascaris. Five specimens of larvae were obtained from the pleural cavity, most of them were surrounded by a group or mass of white blood corpuscles and histiocytes. Many larvae were present in the liver as well as in the abdominal cavity, and also three in the spleen, four in the pancreas and two in the left kidney. All larvae found in these organs were quite as young as those just hatched out in the intestine, in size and in organization of body, measuring from 0.2 to 0.24 mm. in length. Thus the larvae found in the abdominal cavity and in other organs may surely be considered to have pierced through the intestinal wall. This was repeatedly proved by the following experiments.

Exp. 17.—The animal B of the preceding experiment was killed at noon on the 15th, after twenty-four hours. The lungs and liver were infected, three larvae in the pleural cavity, two in the spleen, one in the right kidney, three in the pancreas and many in the abdominal cavity. Larvae were all in the same size as in the preceding case.

Exp. 18.—At 3 p. m., on June 12, two guinea-pigs A and B were fed with ripe eggs sixty-four days old. A was killed at 8 a. m. on the 14th, after forty-one hours. The lungs and liver were heavily infected. Five specimens of larvae in the pleural cavity, many in the abdominal cavity, four in the kidneys, one in the pancreas and none in the spleen.

Exp. 19.—Animal B of Experiment 18 was killed at 10 a. m., on the 14th, after forty-three hours. Result of examination was the same as in the case of A, but two larvae were in the pancreas and three in the kidneys.

Exp. 20.—At 3 p. m., on June 1, three guinea-pigs were fed with the ripe eggs of fifty-two (to A and B) and of fifty-three (to C) days old. C was killed at 3 p. m. on the 3rd, after forty-eight hours. The lungs and liver were infected. Larvae were found in the spleen, in the pleural and abdominal cavity. The pancreas and kidneys were unexamined.

Exp. 21.—Animal B of preceding experiment was killed at 8 a. m. on the 4th, after sixty-five hours. The lungs and liver were infected, twelve larvae being in the abdominal cavity, two in the kidneys, four in the pancreas, while none were in the spleen or in the pleural cavity.

Exp. 22.—At 3 p. m., on June 7, two guinea-pigs (A and B) were fed with the mature eggs, fifty-nine days old; B was killed at 9 a. m. on the 10th, after sixty-six hours. The lungs and liver were infected, the former organ being heavily hemorrhaged. Other organs were unexamined, but the spleen with that of A contained nine specimens of larvae.

Exp. 23.—Animal A of Exp. 22 was killed at 2 p. m. on that day, after seventy-one hours. The lungs and liver were in quite the same state of infection as in the preceding case. Eleven larvae were obtained from the pleural cavity, six of them were in the state of free movement, and the remaining five surrounded by the masses of white blood corpuscles and histiocytes. Eight were in the abdominal cavity, four in the kidneys and two in the pancreas.

Exp. 24.—Animal A of Exp. 20 was killed at 2 a. m. on the 4th, after seventy-one hours. The lungs and liver were heavily infected, two specimens of larvae were present in the pleural cavity, one in the spleen, three in the kidneys and many in the abdominal cavity, but none in the pancreas.

Exp. 25.—At 10 a. m. on June 3, two guinea-pigs A and B were fed with ripe eggs fifty-five days old, they were killed at 1 p. m. on the 6th, after seventy-five hours. In A the lungs and liver were infected, four larvae in the pleural cavity, eight in the abdominal cavity, two in the spleen, one in the pancreas and none in the kidneys. In B the lungs and liver were in the same state as in the case A, a few larvae in the abdominal cavity, two in the kidney, but none in the pleural cavity, spleen and pancreas.

TABLE 2

No.	Feeding Date	Age of Eggs, Days	Killing Date	Hours Passed	Pleural Cavity	Lungs	Ab-dom. Cavity	Liver	Spleen	Pan-creas	Kidney
1	Noon June 14 A	61	8 a. m. June 15	20	5	Bloody spot, many larvae	Many	Many	3	4	2 (left)
2	Noon June 14 B	61	Noon June 15	24	3	Slightly blooded present	Many	Present	2	3	1 (rt.)
3	3 p. m. June 12 A	64	8 a. m. June 14	41	5	Many	Many	Many	None	1	4
4	3 p. m. June 12 B	64	10 a. m. June 14	43	Present	Many	Many	Many	None	2	3
5	3 p. m. June 1 C	53	3 p. m. June 4	48	Present	Present	Present	Present	Present		
6	3 p. m. June 1 B	52	8 a. m. June 4	65	None	Present	12	Present	None	4	2
7	3 p. m. June 7 B	59	9 a. m. June 10	66	......	Heavily blooded present	......	Present	9		
8	3 p. m. June 7 A	59	2 p. m. June 10	71	11	Heavily blooded present	8	Present		2	4
9	3 p. m. June 1 A	52	2 p. m. June 4	71	2	Heavily blooded present	Many	Present	1	None	3
10	10 a. m. June 3 A	55	1 p. m. June 6	75	4	Heavily blooded present	8	Present	2	1	None
11	10 a. m. June 3 B	55	1 p. m. June 6	75	None	Heavily blooded present	Present	Present	None	2	None

Results of these feeding experiments give the facts accurately in the course of the migration carried out by the ascarid larvae in the body of host, as was surely decided in combination with the result of above injecting experiments.

Larvae hatched out in the intestine immediately proceed to the abdominal cavity piercing through the intestinal wall. Larvae in the cavity may wander everywhere freely, that is, some to the liver, spleen, pancreas or kidneys in the cavity and others piercing the diaphragm to enter the pleural cavity, finally penetrating into the lung from its surface. Thus it is believed that the larvae migrate in every direction,

boring through various organs or tissues by means of their own power of piercing, but not by the way of blood vessels.

As to the course of larvae migrating from the intestine to the lung F. H. Stewart supposed two ways and said: "1. Boring through the wall of the stomach or intestine the larva enters a mesenteric venule and is carried to the liver. Thence they pass in the hepatic vein to the heart and by the pulmonary artery to the lung. 2. The larva after hatching in the stomach or duodenum travels by the bile duct and through the degenerated liver tissues and reaching a hepatic venule continues its course as in the first case."

Stewart's supposition might be true, but it would be an accidental case and not the sole or general course of migration, I think. If a blood vessel is the only way by which the larvae migrate in the body of host, the appearance of larvae in the pleural cavity is very difficult to explain.

It was formerly considered by Stewart as well as myself that larvae migrate successively from the intestine to the lung, passing through the liver, in consequence of which the larvae appear in the lungs later than in the liver. From this belief it was easy to think the larvae travel in the blood vessel from the liver to the lung, passing through the heart. But the belief in this successive migration of larvae has been radically destroyed by my above experiments.

The larvae in the abdominal cavity may easily wander about everywhere, and penetrate not only into various organs or tissues in the cavity, but into the lungs, piercing through the diaphragm. Thus the liver, with several other organs in the abdominal cavity, is not a necessary and important organ to be passed for the larvae to reach the lung, as we formerly considered. Larvae penetrating into the liver might be considered to travel to the lungs by the way of a blood vessel, but it is not the general way for larvae to reach the organ. Hence I am inclined to believe that the larvae migrate from the intestine to the lung by their strong power of boring through the various organs or tissues, but not by the way of a blood vessel.

The general and important course of migration by the ascarid larvae in the body of host may be as follows: The ascarid larvae escape from the eggshell in the intestine of host and proceed to the abdominal cavity by boring through the wall of intestine. Thence they pierce the diaphragm to enter the pleural cavity, finally penetrating into the lungs from their surface. It might be considered as an additional and mere accidental course of migration that the larvae in the abdominal cavity penetrate into the liver, thence they are carried to the lungs by the way of blood vessels passing through the heart. This belief in the course of migration of the larvae is strengthened by histological study of the infected organs and tissues. It shows that almost all the larvae in the

lungs and liver are not found in the blood vessels but in other tissues. Details on this side, however, will be reported in another paper.

Furthermore, the lungs are the only necessary and important organ to be passed by the larvae in the course of their development. In this organ the larvae stay longer than in any other, and they develop rapidly there as completely as possible, reaching a length of about 1.7 to 2.0 mm. Larvae in the lungs, several days after feeding, are generally much larger than those in any other organ. This fact was repeatedly observed in recent experiments. The results of some experiments on this subject are given in Table 3.

TABLE 3

Duration of Infestation	Length of Worm in						
	Lungs	Pleural Cavity	Abdominal Cavity	Liver	Spleen	Pancreas	Kidney
162 hrs.	0.3 -0.57 0.95-1.0	0.28 0.48	0.28 0.48	0.28 0.48			
166 hrs.	0.4 -0.51 0.85-1.33	0.28 0.46	....	0.4	0.28	0.28 0.44	0.28
250 hrs.	1.8 2.0	0.3	0.28				

The larvae in the lungs continue their development and migrate to the mouth cavity through the trachea, again passing down the alimentary canal to the intestine of the host.

The occurrence of larvae in the spleen was first mistakenly reported by Stewart in his first paper, and corrected in his second paper. Afterward B. H. Ransom and W. D. Foster recognized the presence of larvae in the organ. I have frequently found the larvae not only in the spleen but in the pancreas and kidneys. The occurrence of larvae in these organs is easily explained by their power in boring through tissues. It should be, therefore, clear that the larvae might penetrate into other organs or tissues such as ovarium, adrenal body, etc., which as yet I have not examined. All these organs and tissues are accidentlly or temporarily visited by the larvae and in them they are not able to complete or continue development. Larvae in the liver, however, might be carried in the hepatic vein to the heart and consequently by the pulmonary artery to the lungs, where they can develop further. The fate of larvae in organs or tissues other than the lung is not yet traced accurately, experiments on this problem being under way. From the results of some experiments, however, I have learned the following facts: After several days (five to ten) after feeding, few larvae or none were found in these organs, but they were more or less present in the abdominal and pleural cavity. The more time passed the more the larvae decreased in number in these organs while the more they increased in the lung. The larvae in these organs were

much smaller than those in the lung, and some of the former were dead or decomposed in structure. So great variation is found in size of larvae in the lung that it cannot be caused by the individual difference only. The larger specimens were three or four times as large as the smaller one, which was in the same size as those in the liver and other organs or tissues. This great variation is probably due to a different invasion of larvae. It is easy to see that the rapid growth of larvae in the lungs may cause great difference in size according to difference in time of invasion.

From these facts we may infer that some larvae in these organs may go back in the abdominal cavity and proceed to the pleural cavity to invade the lungs, the important place for their further development, and those remaining probably perish in these organs. At any rate, the details of larval fate in these organs, except the lung, should be left untouched here.

References Cited

Ransom, B. H., and Foster, W. D. 1917.—Life History of Ascaris lumbricoides and Related Forms. Jour. of Agric. Research, 11: 395-398.

Stewart, F. H. 1916.—On the Life History of Ascaris lumbricoides. Brit. Med. Jour., 2: 5-7.

1916a.—Further Experiments on Ascaris Infection. Brit. Med. Jour., 2: 486.

1916b.—On the Life History of Ascaris lumbricoides. Brit. Med. Jour., 2: 753.

1917.—On the Development of Ascaris lumbricoides L. and Ascaris suilla Duj. in the Rat and Mouse. Parasit., 9: 213-227.

1918.—On the Development of Ascaris lumbricoides and Ascaris mystax in the Mouse. Parasit., 10: 189-196.

1918.—On the Life History of Ascaris lumbricoides. Parasit., 10: 197-205.

Yoshida, S. O. 1919.—On the development of Ascaris lumbricoides L. [also published in Japanese]. Jour. of Parasit., 5: 105-114.

ON THE LIFE HISTORY OF *DAVAINEA TETRAGONA* (MOLIN), A FOWL TAPEWORM *

James E. Ackert

Further experimental studies by the writer on the life histories of fowl cestodes have demonstrated that tapeworms which appear to be *Davainea tetragona* (Molin) may be transmitted to *Gallus domesticus* by feeding them *Musca domestica* Lin. That this fly may be the means of transmitting other fowl cestodes is known. Grassi and Rovelli (1892: 33, 87) found in its body larvae whose scolices closely resembled those of *Choanotaenia infundibuliformis* (Goeze), while Gutberlet (1916: 235) succeeded both in infecting *M. domestica* with cysticerci by feeding onchospheres of this tapeworm, and in rearing adult worms by giving to fowls house flies taken from nature. And the writer (1918: 41) reared *Davainea cesticillus* (Molin) in *Gallus domesticus* by feeding *M. domestica* which some weeks previously had been given onchospheres of this cestode.

In the present experiment, chicks hatched in incubators were taken under cover to one of two experimental feeding houses which had been fumigated ten hours with sodium cyanide and thoroughly cleaned. The cement floors and 18-inch walls excluded worm-like animals, while the screened openings and enclosed vestibules facilitated in eliminating winged forms. On entering the vestibule any intruder was captured before proceeding to the interior of the feeding house. Extreme care was exercised in administering the food, the uncooked portion containing no animal tissues except occasional feedings of fresh beef, and of course, the experimental feedings. By these methods, which have been employed during the last five years, control chicks running with the experimental ones have been free from parasitic worms in every case. It is possible that an occasional arthropod may enter through the fourteen-mesh window screens, but the control chicks have equal opportunity with the experimental ones for ingesting such forms. The chances of such animals being infected with fowl tapeworm larvae are minimized owing to the fact that the experimental feeding houses are 250 yards from a poultry yard.

In September, 1918, several poultry yards in the vicinity of Manhattan, Kansas, were visited. Spring chicks were examined, and at two places *D. tetragona* were among the tapeworms found. In the

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chicken houses and under roosts the freshly voided feces were covered with flies, many of which were *M. domestica.* At these places large fly traps were set near the chicken houses and thousands of flies trapped. From day to day the traps were collected and immersed in tap water for several hours to facilitate identification of the flies. It was found that the latter could be removed from the traps, slightly dried, and the *M. domestica* sorted out one by one before they recuperated sufficiently to escape. They were then given to chicks reared in the experimental feeding houses. When the flies were immersed seventeen hours and then placed by an open window, large numbers recovered fully in two and one-half to three hours after their removal from the water. Among the lots of *M. domestica* given to the experimental chickens were many flies making random movements indicating that any possible tapeworm larvae in their bodies were probably uninjured by the immersion.

Numerous *M. domestica,* obtained in this way, were given as follows to chicks 2½ months old during the autumn of 1918: 3,506 flies to each of chicks 233, 234 and 235 in eighteen feedings, ranging from thirty-nine to 493 between September 23 and October 19; 3,467 flies to chick 236 in seventeen feedings, varying from eighty-nine to 493 between September 24 and October 19; 2,938 flies to each of chicks 239, 240, 241, 242, 243, 245, 246 and 247 in fifteen lots of flies, numbering from 260 to 493 between September 30 and October 19, and 2,026 flies to each of chicks 248, 249, 250 and 251 in thirteen feedings of fifty to 290 from October 4 to October 19.

Early in November, six weeks after the first feeding of flies, four chicks were examined. Two of these were negative, but the other two had mature tapeworms in their intestines, chick 235 having ten long worms, and chick 250 two medium-sized ones, all of which were sexually mature, and some of them possessed gravid proglottids. Two of the control chicks which had been running with the experimental ones were killed, and the examination showed that their intestines were free from parasitic worms. One month later two large tapeworms were found in the intestine of chick 249. During the next eighteen days ten control chicks were examined, and every one of them was free from helminths. In the same period, examinations of the remaining eleven experimental chicks showed that none of these was infected.

Morphological studies of several of the cestodes obtained are here recorded. These tapeworms vary in length from 31 to 307 mm., and from 1 to 3 mm. in width. The scolices measure 247 to 410μ long by 187 to 394μ wide, and are provided with retractile rostella varying from 49 to 57μ in breadth, each armed with a single row of about 100 hooks, 7 to 8μ long. A short dorsal root and a long ventral one

are characteristic of each rostellar hook which is provided with a small, recurved prong. The oval suckers measure 94 to 170μ in longitudinal diameter by 53 to 82μ in transverse diameter, and are armed with eight to ten rows of minute hooks varying in length from 5 to 8.75μ. Of the two short roots, the ventral is slightly the longer, but it is shorter than the prong, which is slender and curved. The neck is long and slender. The proglottids are trapezoidal and imbricate, and the edge of the strobila is serrate. Nearly all of the proglottids are broader than long, the width of the anterior ones being two and one-half to four times the length, that of the middle ones, two to two and one-half times the long axis, and the breadth of the posterior segments, twice the length or about equal to it, depending on the age of the worm. The unilateral genital pores, numbering one in each segment, are situated at or in front of the middle of the lateral margin and are marked occasionally by papillae. The vas deferens and vagina pass on the dorsal side of the nerve, but between the excretory vessels.

Of the male genitalia, the testes, numbering 21 to 25, lie in the median portion, mostly on the aporose side of the proglottid. The vas deferens lies in the anterior third of the segment; it begins near the middle and extends laterally in a much convoluted course to the base of the cirrus pouch which it enters; after two or three coils it becomes continuous with the cirrus which, apparently, is without spines. The pyriform cirrus pouch, measuring 77 to 84μ in length, is surrounded by a layer of muscles 3.5μ thick.

A branched ovary in the middle of the proglottid is the most conspicuous part of the female genitalia. Posterior and slightly to the left of the ovary is the yolk gland, somewhat reniform, with a diameter ranging from 90 to 114μ. The shell gland, lying in front of the yolk gland and dorsal to it, may vary from 52 to 60μ in diameter. The vagina begins at the genital pore posterior to the cirrus pouch; at first it is slender, but at a distance of about 15μ it widens into a thin-walled tube adapted for a seminal receptacle which extends transversely across the proglottid, passing dorsal to the nerve and between the two excretory vessels to the middle of the segment where it bends posteriorly and ventrally, joining the oviduct immediately behind the ovary. After connecting with the vitelloduct in the shell gland, the oviduct extends forward and ends on the dorsal side of the ovary. A persistent uterus is not formed. The eggs pass from the distal end of the oviduct and become imbedded in a fibrous, granular mass which ultimately fills most of the proglottid. This structure divides into 40 to 114 parts, forming egg masses, each of which contains six or more eggs, and is surrounded by a membrane. The individual egg has three envelopes: an inner, surrounding the onchosphere; a middle, some-

what wrinkled; and a smooth, outer covering. The diameter of the onchosphere is approximately 11μ, while that of the outer envelope is about 46μ.

From these studies it seems evident that several of the cestodes obtained are *Davainea tetragona* Blanchard 1891 (in part[1]). Certain variations in structure occur which, hitherto, have not been recorded for this cestode, but they are more nearly in accord with *D. tetragona* than with the somewhat closely related species, *D. echinobothrida* Blanchard 1891. The length of the longest of these cestodes is 307 mm., while the maximum length recorded for either of the above species is 250 mm. Of these tapeworms the maximum width of scolex is 394μ as compared with 350μ given by Ransom (1904: 278) for this worm, and 450μ by the same author for *D. echinobothrida.* The maximum longitudinal diameter of the suckers of the cestodes in question is 170μ, which exceeds the maximum recorded size for this worm 60μ, and lacks 30μ of attaining the greatest diameter of the suckers of *D. echinobothrida,* according to Gutberlet (1916: 36). In these cestodes, the vagina and vas deferens pass between the excretory vessels instead of dorsal to them as is usually recorded for *D. tetragona.* The number of egg masses reported for this tapeworm varies from 50 to 100, but gravid proglottids of the cestodes under consideration may have from 40 to 114 such masses. These small differences in size and structure, in the writer's opinion, should be considered only as variations in the morphology of *D. tetragona.*

The evidence presented points to *M. domestica* as an intermediate host of *D. tetragona,* but thus far larvae of this tapeworm have not been found in the house fly. As the onchospheres are in masses several times the diameter of an embryo, it seemed possible that *M. domestica* might not be able to ingest them. Accordingly, tests were made. Lot 424, consisting of six *M. domestica* were offered fifty egg masses from a live proglottid in a small drop of sweetened water on a glass slide. In three minutes two of the flies took all the moisture, whereupon the slide was examined microscopically, and of the fifty egg masses twenty-nine remained on the slide. In a second similar trial sixteen egg masses were taken, and in two others, six masses were ingested. Moreover, the walls of some of the egg masses had been ruptured by the sucking of the flies which drew out some of the enclosed oncho-

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1. R. Blanchard (Notices Helminthologiques. Mem. Soc. Zool. France, 4: 436) records a double row of about 200 rostellar hooks, and circular suckers for this worm. The latter are clearly oval in all of these specimens. Under ordinary magnification the rostellar hooks appear to be arranged in a double row, but with the aid of an oil immersion objective individual hooks may be seen throughout their length, and their number in a definite arc of the rostellum counted.

spheres. The adaptability of flies' feet for carrying small organisms made a careful scrutiny for the adherence of egg masses imperative. In one case a mass was carried approximately an inch before it fell from the foot of the fly. These tests showed that egg masses of *D. tetragona* can be ingested by *M. domestica,* and that the latter may take separate onchospheres from the egg masses.

Having determined that onchospheres of this tapeworm are ingested by *M. domestica,* two questions arose: Are ingested egg masses or onchospheres regurgitated by the flies and lost? Do the onchospheres pass through the digestive tract of the flies unaltered? It is known that *M. domestica* after gorging itself with liquid food usually regurgitates, forcing out of the crop through the proboscis a portion of this food. The latter frequently takes the form of a bubble adhering to the withdrawn proboscis. At other times the regurgitated drop of liquid is placed on some object and, according to Graham-Smith (1913: 69, 86), it is then gradually taken into the stomach of the fly. In such cases the object is marked by a relatively large, regurgitation spot with a characteristic, light center surrounded by an opaque, marginal ring. These flat regurgitation spots are easily distinguished from the cone-shaped fecal specks of smaller diameter and darker color. In regurgitating it would not be surprising if ingested egg masses or separate onchospheres would be left in the regurgitation spots. To determine this point, small test cages were constructed, every part of which was capable of being examined by the compound microscope. The four walls were made of glass slides, 38x75 mm., the corners being sealed with melted paraffin. The top of the cage consisted of a glass plate 38 mm. square, which was held in place by strips of gummed labels, while a glass slide served as the base of the cage. In these test cages, the house flies behave normally and lived several days.

As the answers to both questions were obtained from the same experiments, the preliminary tests for solving the second question are given here. To ascertain whether or not the onchospheres go through the flies unaltered, it was necessary to learn the length of time required for the passage of food through their digestive tracts. Several tests were made, a typical one being described in detail. Three *M. domestica* (lot 309) taken from a trap baited with vinegar and corn syrup, were placed in one of the test cages where they were given a few drops of sweetened water colored red by the addition of carmine grains. That the flies fed freely was evident from the bright red appearance of the anteroventral portions of their abdomens — the areas immediately below their crops. Each fly regurgitated once. After fifteen minutes the base slide with the remaining red liquid was removed and a clean

one substituted. Two hours and thirty-eight minutes later four characteristic red fecal specks appeared. During the ensuing three hours, six specks, respectively, were defecated, while a maximum of ten were deposited during the seventh hour after feeding. In the next two hours the number of specks in the cage was increased by only five. The tenth hour showed an addition of six faintly red fecal specks, and in the eleventh hour after feeding there were four defecations which contained so few carmine grains that the latter were visible only by aid of a microscope.

To be certain that the red color of the fecal specks was due to the carmine and not to the vinegar and syrup with which the trap had been baited, the fecal deposits from lots 283-303 of *M. domestica,* isolated in lantern globes from the same trap, were carefully examined. Not one of the several hundred specks was colored red, and examination with a lens confirmed the absence of any red particles.

Other similar tests for ascertaining the length of time required for food to pass through the digestive tract of *M. domestica* were made with fresh blood. In these cases, the feces were voided in nine to twelve hours which is approximately the same period as that determined by Gutberlet (1916: 233) in similar tests on flies. On the other hand, sweetened water and carmine grains, before mentioned, were voided in at least two hours and thirty-eight minutes, and in a test with whole, sweet milk and powdered carmine the bright red fecal specks appeared in one hour and fifty-five minutes. The evidence here presented indicates that the time required for liquid food to pass through the digestive tract of *M. domestica* may vary from one hour and flfty-five minutes to twelve hours.

Having ascertained the approximate period of time required for the passage of liquid food through the body of the house fly, attention was directed to the questions of the egg masses or onchospheres being lost by regurgitation, and of their passing through the alimentary canal unaltered. To determine these points, lots 430 and 431, consisting of six and four *M. domestica,* respectively, were isolated in test cages. Gravid proglottids of *D. tetragona* from freshly voided chicken feces were teased in slightly sweetened tap water. In this medium, fifty-six egg masses were offered to lot 430, and ninety to lot 431. The first lot took seven masses, and the second twelve. As each mass contains six or more onchospheres, lot 430 took at least thirty-six of these embryos and lot 431 a minimum of seventy-two. After feeding the egg masses, each lot of flies was transferred to a clean test cage, whereupon the vacated cages were pried apart and every regurgitation spot examined by the aid of a compound microscope. No structure resembling either an egg mass or an onchosphere

was present in any of the spots. At two-hour intervals the flies were transferred to clean test cages, and each fecal speck studied, a mechanical stage being used to locate the fecal deposits. The examination of these specks was continued until the nineteenth hour after feeding the egg masses. In one instance a globular-shaped structure about the size of an onchosphere occurred in a speck, but it lacked hooks and internal membranes.

From this test and from those made on the eggs of *D. cesticillus* which will be reported in a later paper, it seems evident that few, if any, ingested onchospheres of the cestode in question are either lost by regurgitation or passed through the digestive tracts of these flies unaltered. This evidence and the morphological studies on fourteen adult tapeworms obtained by giving common house flies from infected poultry yards to experimental chicks lead the writer to conclude that *M. domestica* may be the means of transmitting *D. tetragona* from one fowl to another.

SUMMARY

1. Flies trapped at poultry yards infested with *Davainea tetragona* (Molin) and other tapeworms were given (many alive) to young chicks reared in a screened house. The food of all controls was free from animal tissues.

2. In two months the chicks were examined; three contained mature tapeworms, several with embryos. The twelve control chicks were free from parasitic worms.

3. Flies eat both onchospheres and egg masses of *D. tetragona*, and neither when ingested are lost by regurgitation, or passed through the digestive tract unaltered.

4. As common house flies from infected poultry yards constituted the only difference between the food given to the experimental chicks and that fed to the control chicks, evidently *D. tetragona* may be transmitted from one fowl to another by *M. domestica*.

Literature Cited

Ackert, J. E. 1918.—On the Life Cycle of the Fowl Cestode, *Davainea cesticillus* (Molin). Jour. Parasit., 5: 41-43.

Graham-Smith, G. S. 1913.—Flies in Relation to Disease, Non-Bloodsucking Flies. Cambridge.

Grassi, B., and Rovelli, G. 1892.—Ricerche embriologiche sui Cestodi. Atti accad. Gionia di sci. nat., Catania (4), 4: 1-108.

Gutberlet, J. E. 1916.—Studies on the Transmission and Prevention of Cestode Infection in Chickens. Jour. Am. Vet. Med. Assn., 2: 218-237

1916a.—Morphology of Adult and Larval Cestodes from Poultry. Trans. Am. Micr. Soc., 35: 23-44.

Ransom, B. H. 1904.—The Tapeworms of American Chickens and Turkeys. Bur. An. Ind. Ann. Rept., 21: 268-285.

ON THE LIFE HISTORY OF THE CHICKEN CESTODE, *HYMENOLEPIS CARIOCA* (MAGALHAES)*

John E. Guberlet

The problem of tapeworm infestation in chickens has received some attention during the last few years. The investigations being carried on at present are chiefly those with regard to the life cycle of the various forms. Of the six species found in the United States three have been demonstrated experimentally. *Davainea proglottina* (Davaine) was transmitted experimentally to chickens through the slug, *Limax cinerus* Lister, by Grassi and Rovelli (1889: 372; 1892: 86). This species has been reported from only a very few localities in this country. *Choanotaenia infundibuliformis* (Goeze) was transmitted through the common house fly, *Musca domestica* Linn., by the writer (Gutberlet 1916a:235; 1916b:30). *Davainea cesticillus* (Molin) has also the house fly, *Musca domestica* Linn., as its intermediate host (Ackert 1918: 41).

Recently, the writer has demonstrated experimentally that the stable fly, *Stomoxys calcitrans* Linn., may transmit to chickens another tapeworm, *Hymenolepis carioca* (Magalhaes 1898). The chickens used in these experiments were hatched in an incubator and placed as soon as coming from the eggs in insect proof cages. Great care was taken in feeding the birds so that no insects entered the cages or were given to the birds with the food. The chicks were fed grain and a small amount of green feed which was carefully inspected.

The experiments were carried on at two different times and the cestodes were obtained from the birds on both occasions by postmortem examinations. In August, 1914, on a farm at Hardy, Nebraska, the writer placed six chicks as soon as hatched into an insect-proof cage. Three were used in the experiment and the remaining three were used as controls. Large numbers of *Stomoxys calcitrans* were taken on August 18, 19 and 20 from around the chicken house and yards and given to three of the chicks. The chicks were all killed on August 29 and two (one was two and one-half weeks old) of the three experimental chicks each harbored seven small cestodes. The other chick as well as the three controls were free from parasites. The writer was compelled to give up the work at that time on account of a change of location and consequently could not carry on the experiments any farther until the autumn of 1918. On December 16-19 seventy-seven

* Contribution from the Parasitology Laboratory of the Oklahoma Agricultural Experiment Station.

flies, *Stomoxys calcitrans,* were given to a chick reared in an insect-proof cage at the Poultry Plant of the Experiment Station at Stillwater, Oklahoma. More flies could not be obtained at this time of the year because of cold weather. This chick was killed on February 11, 1919, and upon examination was found to harbor three mature worms of the species *Hymenolepis carioca.* From the same cage twenty-four other chicks used for other experiments were killed and in no case was there an infection with this species.

At the time when this experiment was carried on in Nebraska the infestation with *Hymenolepis carioca* was very heavy in nearly all of the chickens on the farm and at the same time the *Stomoxys calcitrans* were particularly numerous. At the Poultry Plant of the Oklahoma Experiment Station this species of cestode was not common until in November and December when the chickens became very heavily infested. During this period the *Stomoxys calcitans* also were very abundant about the poultry yards. This was more evident on account of the scarcity of other species of flies. At this season of the year these flies seem to be somewhat sluggish and inactive and consequently become easy prey for chickens.

Large numbers of flies of the species *Stomoxys calcitrans* were fed on onchospheres and fragments of mature proglottids of *Hymenolepis carioca.* The flies during the course of the feeding experiment were fed on milk, syrup and small amounts of sterile chicken droppings which they ate very readily. The flies were kept alive as long as possible and when they died they were preserved for sectioning.

Hymenalepis carioca (Malgalhaes 1898) Ransom 1902

Diagnosis: Length 20 to 110 mm. Breadth at neck 75 to 150μ, at posterior end 0.4 to 0.8 mm. Segments three to five times or more broader than long throughout strobila. Head (Fig. 1) flattened dorsoventrally, 140 to 160μ long, 150 to 215μ wide and 100 to 150μ thick. Suckers shallow, 75 to 100μ in diameter, armed with hooks (Fig. 2) 6 to 8μ in length with short ventral root and dorsal root a mere knob. Rostellum (*r*) unarmed; in retracted position 25 to 45μ in diameter and 90 to 110μ in length with a small pocket (*rp*) opening to exterior in anterior position. Unsegmented neck portion of strobila 0.6 to 1.5 mm. long. Genital pores almost entirely unilateral, a single pore being located in each segment slightly in front of middle of right hand margin.

Male Reproductive Organs: Testicles three in number, normally two on left and one on right of median line. On dorsal side of inner end of cirrus pouch vas deferens is swollen into prominent seminal vesicle (*sv*) which may attain a size of 70 by 50μ. Cirrus pouch (*cp*) in sexually mature segments 120 to 175μ long by 15 to 18μ in diameter; almost cylindrical, slightly curved toward ventral surface of segment;

on outer surface about 20 longitudinal muscle bands, 2 to 3μ in thickness, very prominent in cross section; vas deferens enlarged within cirrus pouch to form small reservoir occupying proximal portion of pouch; distal portion of vas deferens within pouch very slender and functions as cirrus. Genital cloaca 12 to 20μ deep.

Female Reproductive Organs: Opening of vagina in floor of genital cloaca, ventral and posterior to cirrus opening. First portion of vagina very narrow, 1 to 2μ in diameter. Vagina passes inward past excretory canals and in sexually mature segments becomes swollen into prominent seminal receptacle (*sr*) which extends forward to anterior border of segment and inward to proximal end of cirrus pouch. Ovary (*o*) faintly bilobed or trilobed in posterior half of proglottid. Yolk gland (*y*) spherical or ovoid 30 to 40μ in diameter, situated near median line of segment, posterior and dorsal of ovary. Uterus at first a solid cord of cells extending transversely across segment along anterior border of ovary; becomes hollowed out and grows backward on dorsal side of ovary; in gravid segments (Fig. 4) occupies nearly entire segment and filled with eggs. Embryos (Fig. 5) in gravid uterus spherical or oval, with four membranes, the two middle membranes often approximated to form thick layer which shows a somewhat granular structure. Diameter of outer membrane 38 by 38μ to 80 by 75μ, of outer middle membrane 32 by 32μ to 70 by 65μ, of inner middle membrane 26 by 26μ to 45 by 40μ, of inner membrane 24 by 18μ to 32 by 24μ. This membrane lies so close to onchosphere that it is almost impossible to distinguish it from embryo. The embryonic hooks penetrate this membrane. The onchosphere is 22 by 16μ to 30 by 22μ in diameter; onchospheric hooks (Fig. 6) are 8 to 10μ in length.

This thread-like worm, according to the above observations, seemed to be most numerous during the late summer and fall at the seasons of the year when *Stomoxys calcitrans* are very abundant. During the autumn this species of fly is less active and consequently is more easily taken by chickens. Experimentally infesting chicks with *Hymenolepis carioca* through feeding infested stable flies *Stomoxys calcitrans* under control conditions makes it evident that this species of fly may be the intermediate host of this species of chicken cestode.

LITERATURE CITED

Ackert, J. E. 1918.—On the Life Cycle of the Fowl Cestode, *Davainea cesticillus* (Molin). Jour. Parasit., 5: 41-43.

Grassi, B., and Rovelli, G. 1889.—Embryologische Forschungen an Cestoden. Centralbl. Bakt. und Parasitenk., 5: 370-377; 401-410.

1892.—Richerche embriologiche sui Cestodi. Att. Accad. Gioenia Sci. Nat. in Catania, 4: 1-108.

Gutberlet, J. E. 1916.—Studies on the Transmission and Prevention of Cestode Infection in Chickens. Jour. Am. Vet. Med. Assn., 49: 218-237.

1916.—Morphology of Adult and Larval Cestodes from Poultry. Trans. Am. Mic. Soc., 35: 23-44.

EXPLANATION OF PLATE

Fig. 1.—Scolex of *Hymenolepis carioca,* × 160.
Fig. 2.—Hooks from suckers. Free hand drawing, × about 2000.
Fig. 3.—Reconstruction of proglottids showing organs, × 200.
Fig. 4.—Section of ripe proglottids showing gravid uterus, × 40.
Fig. 5.—Embryos with membrane, × 600.
Fig. 6.—Onchospheric hooks, × 600.
Drawings made with aid of camera lucida.

Abreviations

cp — cirrus pouch
dex — dorsal excretory canal
vex — ventral excretory canal
o — ovary
r — rostellum
rp — rostellar pocket
sr — seminal receptacle
sv — seminal vesicle
t — testes
y — yolk gland

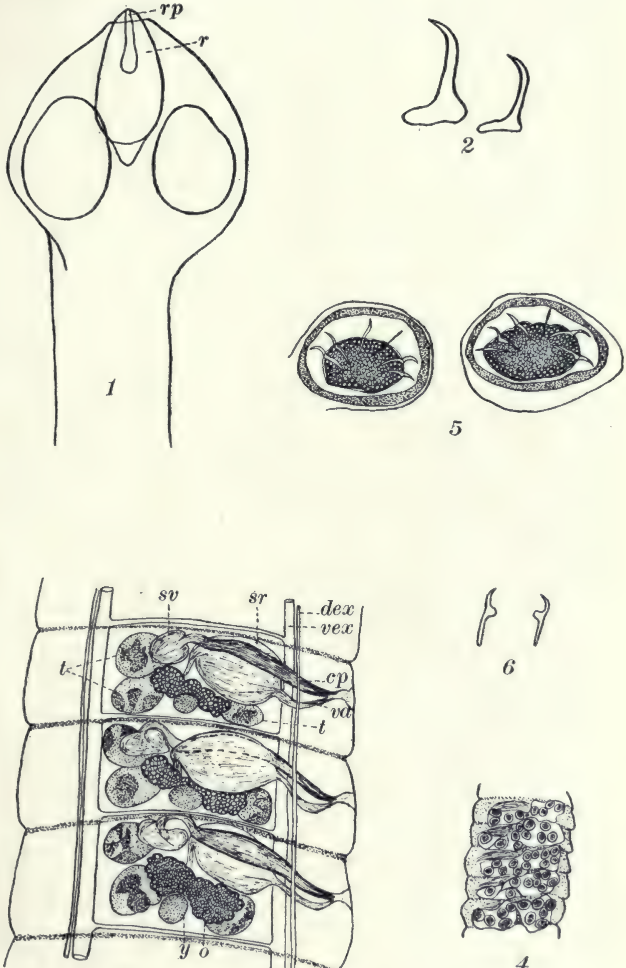

PLATE IV

FURTHER NOTES ON THE STUDY OF THE HUMAN LUNG DISTOME, *PARAGONIMUS WESTERMANI*

KOAN NAKAGAWA

(From the Government Hospital, Taichu, Formosa)

I. CERCARIA FOUND IN FRESHWATER SNAIL

Since 1915, when I first discovered seventeen species of cercariae infesting the mollusks found in the rivers in Shinchiku Prefecture, Formosa, I have attempted to infest with the miracidia of the human lung distome the river snails, in which the miracidia of other species can develop into cercariae, and thus to learn the complete life cycle of the parasite in question. I thought that one of the seventeen different forms of the cercariae I examined belonged to that of the human lung distome for the following reasons:

(a) This is the only form found in the aboriginal villages, where human lung distomiasis is most prevalent.

(b) Both the miracidia of the human lung distome and this species of cercaria prefer a particular water snail to others as hosts.

(c) Similarity in shape of the spine in the oral sucker and of the excretory vesicle of both the encysted larvae of the human lung distome in the crab and this species of cercariae.

To prove the above conjecture a great many devices were tried, but all turned out to be futile, due probably to the lack of good tap water. It was very difficult to keep the water-snail alive in the aquarium long enough to finish the infection experiments. Finally a live box was made and immersed in the river. In this box both the water snails having the supposed cercariae of the human lung distome and the crabs free from previous infection were put together. This experiment also failed. But I found in the crabs the youngest encysted larvae that seems to have just entered. They were supposed to be those of the human lung distomes, since similar larvae have been reported from Japan proper—Niigata, Gifu, Okayama and Tokushima prefectures. So I reported elsewhere the encysted larvae I found were those of the human lung distome.

In the spring of 1917, Dr. Yokokawa and myself discovered a new species of the encysted larvae infesting the crabs found in the infected regions of Shinchiku Prefecture in Formosa. They were identified to be those of *Stephanolecithus parvus* n.g., n.sp., that is a species independent of the human lung distome. It may be objected that there may be more than one species of cercariae infesting the water snails found in Japan proper, and mine may not actually be those

of the human lung distome. To meet this objection investigation was resumed in October, 1917, at Kalapai. To my great astonishment, there I could find four different species of cercariae. No. 12* (80.0 per cent), No. 15 (13.3 per cent.), No. 4 (3.3 per cent.), and the newly discovered one (16.6 per cent.). Besides, I came across two forms of redia, of unknown species. Usually only one species of cercaria is found in the water snails, but there are also many cases in which more than one species of the cercariae are found in one individual. This newly discovered cercaria seemed more closely related with that of the human lung distome than No. 12 does.

The cercaria No. 12 was discovered by myself in January of 1918, widely distributed in the water snails found in the rivers running through the villages free from infection, such as Ako, Tainan, Kagi and Nanto. This makes their identity to the human lung distome extremely doubtful. The newly discovered cercaria will therefore be described in some detail.

This cercaria was discovered in May, 1917, by myself in *Melania libertina* G. found in the rivulet in Torunsho in Shinchiku Province, and in December of the same year, in the same species of the water snail in Kalapai. It is oblong, 0.2 to 0.25 mm. long by 0.08 to 0.1 mm. wide. The oral sucker is large, its diameter being 0.06 mm. It is provided with a sharp spine. The abdominal sucker is smaller than the oral and has the diameter of 0.04 mm. Around the sucker is a group of glandular cells; the glandular ducts run toward the anterior end of the body with a wavy course. The excretory vesicle is a straight tube appearing like a slit and lies on the median line arising very closely to the abdominal sucker and running toward the posterior end of the body. The tail is very small, having the length of 0.02 to 0.03 mm. and the breadth of 0.01 to 0.015 mm. At the posterior end of the tail are several short spines arranged in a row. The cercaria moves fairly lively.

The redia which gives rise to the cercaria was also found. Young rediae are spheroidal, 0.1 to 0.2 mm. in diameter, or spindle shaped, 0.2 mm. long by 0.1 mm. wide. The full grown rediae may either be spindle shaped or cylindrical, with the length of 0.3 to 0.7 mm. by the width of 0.15 to 0.3 mm. They have a pharynx 0.1 mm. in diameter and a voluminous intestine, which reaches as far as the posterior margin of the body. In the intestine is found a brownish or variegated mass.

Morphologically this species is more closely related to the young encysted larva of the human lung distome found in the crab than

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* The cercariae which came to my observation have been provisionally called by numbers.

No. 12, which has been reported as the cercaria of the human lung distome in the Journal of Experimental Medicine, vol. 26, No. 3. This new discovery has already been reported in Japanese in the Tokyo-Iji-Shinshi, February, 1918.

Since that date, various workers published their views regarding this species of cercaria. Kobayashi reported in Japanese the results of his study on the cercaria of the human lung distome carried out in Corea in the Chosen (Corean) Igakkai Zasshi No. 21. He thinks that the cercaria A, as he names it, which is found in *Melania gottschei* M., *Melania nodiperdo* var, *quinaria* M. and *Melania extensa* (?) M., is the cercaria of the human lung distome. It seems very likely that this species of cercaria is identical with mine. Besides, Miyairi, who is studying the first intermediate host of the human lung distome, holds

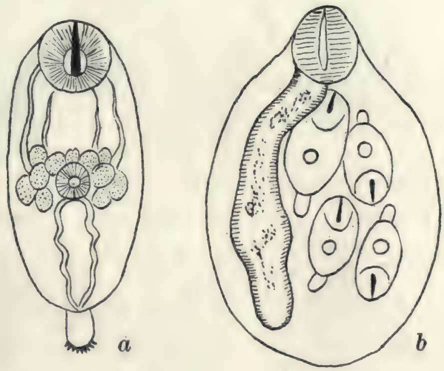

Fig. 1.—Human lung distome; *a*, cercaria, × 160; *b*, redia, × 80.

the view that this species, which is found in *Melania pannicicinca* M. and *Melania extensa* M., must belong to the cercaria of the human lung distome. It was reported also in Japanese by him in the "Ikaijiho," No. 1252, that he had succeeded in obtaining this species experimentally by infecting the water snails with the miracidium of the human lung distome.

Yoshida's cercaria H seems to belong to this species. He states that his cercaria H is provided with the pharynx being situated just in the middle or a little anterior to the middle part of the oral and the abdominal suckers, but in our cercaria it is lacking. Neither are the spines found at the end of the tail. These differences may be due to a difference of observation. Yoshida, however, does not attempt to institute any relation of this species to the cercaria of the human lung distome (Osaka Igakkai Zasshi vol. 16, No. 3).

II. YOUNG ENCYSTED LARVAE FOUND IN THE CRAB

The young encysted larvae in the crab hitherto supposed to be those of the human lung distome as reported in my former communication in the Journal of Experimental Medicine, vol. 26, No. 3, was since identified by Dr. Yokokawa and myself to belong to an undescribed species of parasite, *Stephanolecithus parvus*. Ever since, I have carried out investigation directed toward the discovery of those of the human lung distome in the crab, and I think I have succeeded in doing this.

This form is chiefly found wedged in the muscular tissues or in the epidermis of the crab. Its shape and size vary according to the ages of the worm.

The youngest specimens move freely through the tissues of the host, showing a squirming motion in a thin cyst wall. The cyst is very

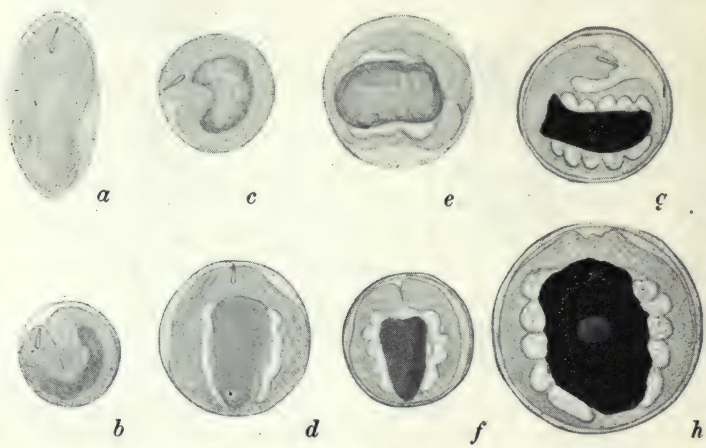

Fig. 2.—Encysted larvae; *a*, youngest found, × 120; *b-e*, successive stages, × 80; *f*, nearly full grown, × 80; *g-h*, full grown, × 80.

pliable and changes its shape according to the worm inside. The stretched specimen together with the cyst measures 0.18 to 0.26 mm in length by 0.11 to 0.1 mm. in breadth. The oral sucker is provided with a sharp spine. The abdominal sucker is smaller than the oral. It lies a little anterior to the middle part of the body. The excretory vesicle is slit-like, being situated on the median line arising from the dorsal side of the abdominal sucker or sometimes beyond the sucker and reaches as far as the end of the tail. The intestine has not yet developed.

Some of the encysted larvae have a globular shape, 0.18 to 0.22 mm. in diameter. The larva lies in the thin wall of the cyst, folded on itself. The excretory vesicle is well developed. It reaches a little

anterior beyond the middle part of the body, and has a dark gray color. The suckers are just the same as those just described.

The large specimen has the diameter of 0.24 to 0.26 mm. The wall of the cyst is thin and pliable. The worm lies folded on itself or sometimes straight. The excretory vesicle is very large, and has the content consisting of coarse granules of a gray color. The excretory vesicle is especially thickly provided with pigment. A slender, long and winding intestine lies laterally along the excretory vesicle. It is somewhat difficult to detect it. The encysted larvae are sometimes oblong in shape. The cyst wall is so thin that slight pressure breaks it, liberating the worm.

The young distome just out of the cyst has a leaf-like form 0.3 mm. long by 0.15 mm. wide. The oral sucker has the diameter of 0.04 mm., and is provided with one spine. The pharynx is well developed. The esophagus is short. The intestine is slender and takes a slightly winding course laterally to the excretory vesicle. The abdominal sucker is smaller than the oral, and measures 0.035 mm. in diameter. It is situated a little anterior to the middle part of the body. The surface of the worm is covered with short, weak spines.

The encysted larvae are found both in the muscular tissues and the epidermis of the crab. Usually we come across very few of them. The reason for this is probably that the encysted larvae of this species are not conspicuous and are likely to be overlooked, being taken for the section of the muscular tissues or deposition of the cuticular pigment. The thinness of the wall of the cyst may make its detection very difficult. In one of the crabs kept for two weeks in confinement I found a very large number of the fairly well developed encysted larvae. Outside the muscle and the epidermis, they were also found in the liver, where their detection is most difficult. They have not yet been discovered in the gills.

This species of the encysted larvae appears very different from the full grown encysted larva of the human lung distome, but close examination will reveal that they decidedly show characteristic developmental stages of the human lung distome. I do not hesitate to state that its identity with the larvae of *Paragonimus westermani* is a matter beyond dispute.

DISSOTREMA SYNONYMOUS WITH GYLIAUCHEN

SEITARO GOTO
Imperial University of Tokyo

In a recently published paper (Goto and Matsudaira, 1918) I described jointly with the late Mr. Matsudaira an amphistomatid parasite as a new genus and species under the name of *Dissotrema papillatum.* A search of some further literature to which I was able to gain access later showed, however, that a worm had been described by Nicoll in 1915 under the name of *Gyliauchen tacharodes,* with which my species presents so many points of close resemblance that the two should be placed in the same genus. Dissotrema therefore becomes by the rule of priority a synonym of Gyliauchen.

Gyliauchen tacharodes was found in considerable numbers in the intestine of a pilot fish (*Tachysurus* n. sp.). The body measured 2.6 to 3.5 mm. in length, was elongated, moderately plump and somewhat pointed at both ends. Cuticula entirely smooth. Oral sucker globular, sub-terminal, 0.24 to 0.26 mm. in diameter; posterior sucker almost at the end of the body, somewhat elongated, 0.50 by 0.47 mm. Prepharynx extraordinarily long, passing down on the right side of the body to the level of the pharynx, where it bends at right angles and passes over to the left side of the body; here it again bends at right angles and proceeds toward the front end of the body, but again bends abruptly backward about midway between the oral sucker and the pharynx and proceeds along the median line of the body to join the pharynx. This extremely muscular organ is 0.28 to 0.35 mm. by 0.23 to 0.27 mm. in size and lies between the first and second third of the body. Intestinal caeca arising directly from the pharynx, short, widely dilated, somewhat horse-shoe shaped, terminating at the level of the ovary, about two fifths of the body length from the posterior end. Genital aperture median, just behind the intestinal bifurcation, some distance in front of the middle of the body; cirrus pouch stout, muscular, ovoid, 0.35 by 0.25 mm. in size, enclosing an almost globular pars prostatica of moderate size and a rather wide ductus; vesicula seminalis L-shaped with the lower limb directed toward the right, immediately following the pars prostatica, considerably larger than the cirrus pouch; testes usually in an oblique pair, a little in front of the acetabulum, usually overlapping a little, almost globular, 0.3 mm. in diameter. Receptaculum seminis globular, nearly as large as the testes, immediately in front of them and in the left half of the body; ovary

much smaller than the receptaculum seminis, immediately in front of it but more toward the median line of the body, usually almost contiguous with the vesicula seminalis. Vitellarium rather scattered and irregular, situated for the most part laterally over the intestinal ceca, not extending beyond the latter posteriorly but anteriorly reaching a little in front of the pharynx; follicles small; yolk ducts running down from the posterior end of the vitellarium on each side, passing round the outer edges of the testes and then turning forward to unite between the testes. Uterus containing very few ova, which are light yellow in color and 78 to 84 by 45 to 49μ in size. No statement is made about the excretory or the lymph system.

A comparison of the two species *tacharodes* and *papillatus* enables me to formulate the generic diagnosis more accurately than was possible in my former paper.

Genus Gyliauchen. Body plump, lightly attenuated at the front end, broader and more rounded at the hind end. Cuticula smooth. Oral sucker globular or ellipsoidal, close to anterior end; acetabulum subterminal, opening on the ventral surface by a longitudinally elongated aperture. Genital opening in the ventral median line in the middle third of the body. Testes subglobular, in oblique pair, directly in front of acetabulum. Ovary subglobular, some distance in front of the testes, median or submedian, much smaller than the testes. Receptaculum seminis globular or flask shaped, subequal to testes in size or notably smaller. Vitellaria lying for the most part in the anterior half of the body, in small, isolated follicles; paired yolk ducts proceeding backward from the hind end of the vitellaria. Cirrus pouch muscular, ovoid, conspicuous. The whole genital organs, with the exception of the vitellaria, lying for the most part in the posterior half of the body. Prepharynx very long and convoluted, terminated by a muscular pharynx; intestinal ceca short and wide, extending only for a short distance into the posterior half of body. Excretory vesicle elongated bottle-shaped, opening by its slender end near the hind end of body; paired excretory vessels proceeding forward from the antero-lateral corners of the vesicle.

Nicoll does not mention the cirrus glands, nor the prepharyngeal glands, but there is a suggestion of the presence of the former in his figure, while as to the latter there is no doubt in my mind that they will be detected on a closer examination.

Concerning the affinity of *G. tacharodes* Nicoll says that it undoubtedly belongs to the family Paramphistomatidae, but that it is difficult to include it in any of the subdivisions of that family, though most closely, albeit remotely related to the Pseudocladorchis of Daday. I

pointed out in my former paper certain resemblances and differences between my parasite and the Paramphistsomatidae and concluded that they were such as to make it more a matter of convenience than of principle whether to refer it to that family or a new one, but that the erection of a new family was more desirable. A classification of the Amphistomatidae has been recently attempted by Stunkard, but it is an admittedly provisional one to be replaced by a more natural system when such is proposed. Now there is one aberrant genus of this family to which I ought to have paid more attention when discussing the affinity of my parasite, and that is Balanorchis (Fischoeder), the exact rank of which Stunkard leaves undecided. In this genus the testes lie in an asymmetrical pair directly in front of the acetabulum and posterior to the ovary, and what is of equal importance is the presence of a cirrus pouch, although its wall appears to be relatively quite thin. If this parasite lost its buccal pouches and perioral papillae, shortened its intestinal ceca and acquired a pharynx at the termination of its long esophagus a form closely similar to Gyliauchen would result. More distantly related to Gyliauchen appears to me to be the parasite described by Nicoll immediately before *G. tacharodes* under the name of *Opitholebes amplicoelus,* from the intestine of *Sphaeroides lunaris,* which the author considers to be not an amphistome but an anomalous distome. If this worm had a long, winding prepharynx and shorter intestinal ceca it would not be very unlike Gyliauchen, altho we do not know anything at present about its excretory system or its possession of a lymph system. Opistholebes appears to be related in turn to Nicoll's *Maculifer subaequiporus* also described in the same paper from the intestine of *Sphaeroides multistriatus.* This is a true distome and related, as Nicoll points out, to the Allocreadiidae; but if it had its acetabulum transferred to near the hind end of the body a worm would result, which is quite like Opistholebes except for the muscular ring of the prepharynx, the systematic significance of which it is difficult to evaluate at present. One more aberrant distome I may mention in this connection and that is the species described by Monticelli as *D. fractum* Rudolphi. It has a long, winding prepharynx surrounded in its entire course by numerous unicellular glands which the author calls "salivary," and which are evidently analogous to the prepharyngeal glands of *Gyliauchen papillatus;* in other respects, however, this distome stands quite remote.

These brief observations serve to show that Gyliauchen stands intermediate, when single characters are compared, between the Paramphistomatidae and some of the aberrant distomes. That it is sufficiently distinct from the former to justify the erection of a new family has been pointed out.

Papers Cited

Daday, E. v. 1907.—In südamerikanischen Fischen lebende Trematoden-Arten. Zool. Jahrb., Syst., 24: 469-590.

Fischoeder, F. 1903.—Die Paramphistomiden der Saugetiere. Zool. Jahrb., Syst., 17: 485-660.

Monticelli, F. S. 1893.—Studii sui Trematodi endoparassiti. Zool. Jahrb., Suppl. 3.

Nicoll, W. 1915.—The Trematode Parasites of North Queensland. III. Parasites of Fishes. Parasitology, 8: 22-40.

Stunkard, H. W. 1917.—Studies on North American Polystomidae, Aspidogastridae and Amphistomidae. Ill. Biol. Monogr., 3, No. 3.

Goto, S., and Matsudaira, Y. 1918.—On Dissotrema papillatum, n. g., n. sp. an Amphistomoid Parasite from a Marine Fish. Jour. Coll. Sc., Imp. Univ. 39, art. 8.

NEW HUMAN PARASITES

Dicercomonas Chalmers and Pekkola, 1919 (not Diesing, 1856). *Dicercomonas soudanensis* Chalmers and Pekkola, 1919.—The proposed new species represents a new genus of flagellates of the family Tetramitidae and is characterized by the absence of cytostome and contractile vacuole, and presence of a simple nucleus, and two anterior and one posterior flagellum, the last being attached to the body for a portion of its length, but ending freely. The species in question was found in fluid feces in a few cases of diarrhea in Khartoum. The zoological position of this flagellate is discussed and a diagram illustrating the relationships of the genera and subfamilies of the Tetramitidae is given, also a key for distinguishing *Dicercomonas* and other genera comprising the subfamily Embadomonadinae. [*Dicercomonas* Chalmers and Pekkola is a homonym of *Dicercomonas* Diesing, 1856, and hence if recognized as a distinct genus must be renamed—B. H. R.] (J. Trop. Med. & Hyg., 22: 29-30; 1 pl., Feb. 15, 1919).

Ornithodorus marocanus Velu, 1919.—This new tick from North Africa is readily distinguishable from *O. erraticus* (Lucas), also a North African species, but is very similar to *O. turicata* (Dugès), an American species. It clearly differs from the latter, however, in certain details of the legs and cuticle. It attacks humen beings and pigs, its bite is painful, and gives rise to a pronounced local reaction of the skin which lasts for several days, sometimes accompanied by fever (Bull. Soc. Path. Exot., 12: 99-104, 9 figs.).

Oncocerca caecutiens Brumpt, 1919.—This species of nematode from Guatemala, which is described and figured by Brumpt, closely resembles *Oncocerca volvulus*. It occurs in subcutaneous tumors usually located on the head. According to Robles (Bull. Soc. Path. Exot., 12: 442), it is the cause of a disease known as coastal erysipelas. In some localities as many as 97 per cent. of the population may be infested with this nematode, Indians more commonly than white, and children and adult males more commonly than adult females. As a rule, only field laborers are affected. Robles thinks that certain species of *Simulium*, which are common in the localities where the parasite is found, serve as vectors (Bull. Soc. Path. Exot., 12: 464-473; 5 figs.).

NOTE

Hookworm disease is a serious matter in Queensland according to Dr. Lambert, who has been investigating for the Rockefeller Foundation. Not less than 23 per cent. of the population of the coast are infected, and if the progress of the disease is not arrested serious degeneracy may be expected in a few generations. Despite denials from political officers statements in the report are confirmed by abundant evidence from scientific sources.

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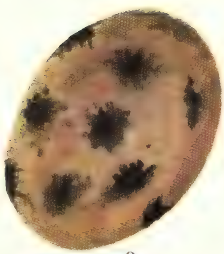

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PLATE V

EXPLANATION OF PLATE

Notropis anogenus Forbes bearing cysts of *Myxobolus aureatus* in the fins. The cysts in life correspond in color to Japanese gilding. Drawn from life by Mrs. H. S. Jennings, Put-in-Bay, Ohio, August 15, 1898.

The Journal of Parasitology

Volume 6 DECEMBER, 1919 Number 2

NOTES ON NORTH AMERICAN MYXOSPORIDIA *

Henry B. Ward

In this paper are published data on three new species of Myxosporidia. Two species were observed in Lake Erie as parasites of a minnow; the third came from a species of Pacific salmon. Both cases present some unusual features that seem worthy of record. I am greatly indebted to my colleague, Dr. R. Kudo, for valuable assistance given while I was working up the material. The beautiful sketch (Plate V) illustrating the species from Lake Erie was prepared by Mrs. H. S. Jennings, to whom my thanks are due for the courtesy.

Myxobolus aureatus nov. spec. (Plate V)

Host: *Notropis anogenus.*
Location: between the fin membranes.
Locality: near Put-in-Bay, Lake Erie.

Some years ago while engaged in the study of fish parasites for the U. S. Bureau of Fisheries, I discovered a case of infection with a sporozoan parasite which, on examination, proved so unusual in character that careful studies were made of the material then available. The notes made at that time were laid aside in order to secure further specimens and to work out the entire life history. It has proved impracticable as yet to repeat the study of fresh material on the spot and the importance of the find leads me to prepare the data for publication in order that the attention of others may be directed to the species. The form studied departs in some respects from all Myxosporidia yet described and commands attention for certain peculiar biological features.

In August, 1898, while I was seining near the hatchery of the U. S. Bureau of Fisheries at Put-in-Bay, Ohio, some minnows were taken which attracted immediate attention by virtue of their striking appearance. Several species of Notropis were netted in the same locality and all were carefully examined. One was conspicuous and only that one was infected in any way. The species in question was

* Contributions from the Zoological Laboratory of the University of Illinois, No. 145.

determined by Dr. W. C. Kendall, who was serving as ichthyologist of the party, as *Notropis anogenus,* although he noted that these individuals were young and did not agree in all details with the description of that species. These minnows were 2 to 3 centimeters long measured without the caudal fin. In all, thirty specimens of this minnow were captured and seven of these were infected with a myxosporidian parasite. The infected specimens were not inferior in size or vigor to the others of the same species. Even those most severely attacked by the parasite manifested normal activity and responded to experimental stimuli as promptly and accurately as those which showed no sign of being infected. The specimen which was most heavily infected was the most vigorous of all the minnows taken. It lived more than twenty-four hours in a small dish only 4 inches in diameter without any change of water and when killed was still very active.

The infection was markedly conspicuous. At first glance one could see one to many small cysts in the membrane of the fins. They lay between the ectodermal layers of the fin membrane, appearing as brilliant opaque points in the otherwise delicately transparent organ. The cysts were particularly conspicuous because of their striking coloring. Each appeared as an oval body perfectly opaque and glittering like a mass of metallic gold. These cysts were absolutely confined to the fins. Nowhere else on the surface of the body could there be seen even a single such structure and careful dissection failed to disclose any in the flesh elsewhere. Nor were any structures found which could be associated with them even as modified cysts or as developmental stages of the organism. This single stage in the location designated was the only phase in the life history of the organism that I was able to discover. Of the unique character of the location and the color, I shall say more later.

The number of such cysts in the individual case varied widely. In one specimen only a single cyst was present. That was located in the anal fin. In most specimens the cysts were fairly numerous. The individual represented in the plate (Fig. 1) shows the average frequency of infection. It had about thirty-five cysts, distributed as follows: two cysts in the dorsal fin, four in the caudal, eight in the anal, four and two in the two pectoral fins, and three in the single ventral fin present, one of the ventrals being missing in this specimen. The most heavily infected individual had about forty cysts; six of these were located in the dorsal fin, five in the anal, ten in the left pectoral and six in the right pectoral, five in the left ventral and seven in the right ventral. The various specimens showed most distinctly that no uniformity of distribution obtains either with regard to the degree of infection in any particular fin or in respect to the fins infected. Careful

examination of the specimens showed that both the paired and the unpaired fins were infected; the right and left sides proved to be variably infected and any one of the fins might be free from infection although the others were at the same time heavily infected.

In most cases the cysts were clearly separated from each other, though in a few instances they were apparently connected. Even here careful examination of the region under appropriate magnification demonstrated the fact that cysts overlapped in profile only and were actually separate from each other. The cysts were usually single and well separated from those nearest, but in some cases a fin carried a group of two to six cysts rather closely grouped together. There seemed to be no regularity in the occurrence of these groups and as already indicated they were in reality separate cysts though appearing on superficial examination to form a connected mass. As they increased in size the cysts seemed to accumulate chromatophores on the surface. At an early stage when the cyst was small, the chromatophores were few in number; later as the cyst increased in size the chromatophores became much more numerous, and in the largest they were thickly strewn over the surface. Such differences were often seen in adjacent cysts in the same group where the mass of chromatophores imparted a darker, heavier aspect to the older cyst.

The examination of these cysts under a higher magnification showed some interesting structural features. They were exceedingly regular in form and fairly uniform in size although the latter appeared to vary a little with age and development. The individual cyst was a smooth margined ellipsoid, measuring from 1 to 1.6 millimeters in larger diameter and from 0.8 to 1.2 millimeters along its transverse axis (PlateV). The striking color of the living cysts has already been mentioned. Under a high power it seemed to be a clear orange yellow, but under all circumstances was perfectly opaque. The surface of the cyst was spotted with conspicuous black patches of minute size. These spots lay on the outer surface of the cyst wall and were in reality the chromatophores of the skin, but they were distinctly more abundant here than elsewhere in the fin or on the body skin of the minnow. The gilt color was contained in the cyst wall itself, as was easily demonstrated on pulling the structure to pieces. This color faded slowly in alcohol and formol, first losing its brilliancy and later disappearing entirely, leaving the cyst wall a dull white or grayish tone. The cyst wall was noticeably tough and thick in spite of the insignificant size of the cyst. When the wall of the living cyst was torn apart by needles, there exuded a milky white mass from the interior consisting chiefly of the spores to be described later. The gilt color and opacity of the wall remained unchanged.

The presence of color is very unusual in myxosporidian cysts. So far as I can ascertain it is shared by no other species yet described. Recently Southwell and Prashad (1918) reported a cyst of *Myxobolus nodularis* in the muscles of *Rasbora daniconius* as of a creamy yellow color, "in one case appearing blackish owing to the large number of black granules scattered in its surface." These granules are very probably chromatophores on the surface and belong to the host tissue as is indicated in descriptions of other species by various authors; if this inference be correct their record is in part similar to that described here. But the color can hardly be comparable. In fact, as the authors just quoted describe cysts of another species (*Myxobolus rohitae*) in the gills of *Labeo rohita* as "of a creamy yellow color," it seems as if they were describing a shade or tone in the preserved specimen rather than a distinct color or pigment; moreover, there is nothing to indicate that the color belongs to the cyst and not to the contents or to the host tissue.

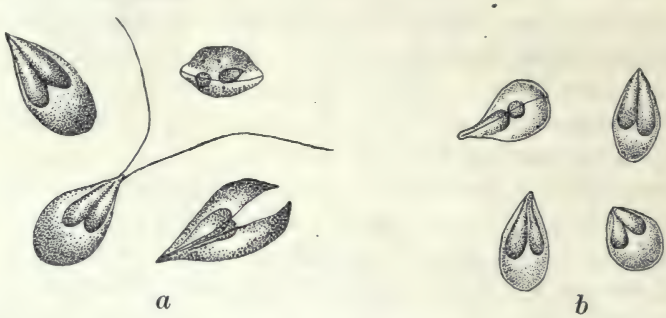

Fig. A.—Spores of *Myxobolus aureatus* drawn from fresh material. *a*, $\times$ 1300; *b*, $\times$ 972.

In discussing such structures various authors agree in stating that the color of the cyst belongs to the host tissues and can be found on examination to disappear when the cyst is removed, thus showing that the true cyst wall is colorless. That is certainly not the condition which obtains in this case, for the color belongs to the cyst and cannot be separated from it in life. In section, the protoplasm shows a poor differentiation into ectoplasm and endoplasm. The former, granular and recticular, covers the entire surface as a thin layer, while the latter is highly vacuolated, containing only mature spores.

When the cyst wall is torn open there exudes a milky white mass composed primarily of the spores. These are characteristic in appearance and demonstrate immediately the myxosporidian nature of the cyst. The spores are ovoid in form (Fig. A), slightly pointed at one end and rounded at the other. The pointed end is the capsular pole. There is no caudal filament present. From one aspect the spore appears

slightly narrower and more pointed than when seen at right angles. Up and down the narrow aspect the spore shows a distinct ridge which marks the line of separation between the two valves of which the spore wall is composed. When the material is left standing in water, the valves separate along this line (Fig. *A, a*) and are seen to be perfectly symmetrical and similar in all respects. The shell is of moderate thickness and bears a flange at the lower non-capsular pole. The greatest convexity of the valve is located two-thirds of the way from the pointed pole. The spores vary in length from 12.4 to 13.5μ with a breadth of 6.5 to 7.5μ, and an average thickness of 5μ.

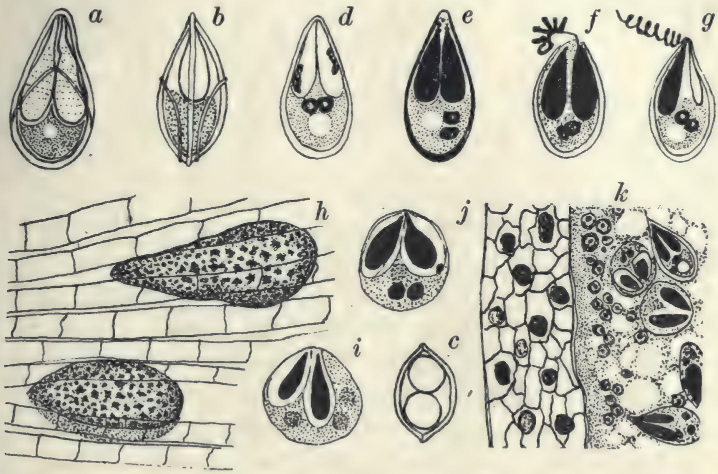

Fig. B.—*Myxobolus aureatus;* magnified 1,500, except as otherwise stated. *a, b, c,* unstained preserved spores in different views; *d, e,* stained mature spores; *f, g,* spores with extruded coiled polar filament from section; preparation stained with Giemsa; *h,* a portion of the caudal fin showing two cysts, $\times$ 22; *i, j,* young spores, stained; *k,* a portion of the cross-section of the fin, showing the peripheral part of the parasite, $\times$ 900.

Each spore contains two capsules, located in the pointed half of the shell. They are not always exactly alike, for frequently one is slightly longer than the other or else located a little further from the actual pole so that its inner end lies in a different plane from the other. These capsules are elongated pyriform in outline and measure 6 to 7 or rarely 7.5μ in greatest length. Ordinarily the filament is not extended, but it can be made to appear by letting the spore stand twenty-four hours or more in plain water. Then one sees two extremely delicate threads, one from each capsule, extending into the water a distance of one and one-half to two times the major diameter of the spore (Fig. *A, a*). In the preserved material they may be forced out (Fig. *B, f, g*) in such fashion as to indicate six or seven coils in the filament. The binucleated

finely granular sporoplasm shows always an iodinophilous vacuole which becomes distinctly contoured by taking a deep brownish color when the spore is treated with Lugol's solution. Its diameter is about 2μ.

The characteristics of this species as described above cause it to fall clearly within the family of the Myxobolidae of Thélohan and the absence of any caudal filament on the spore membrane places it in the genus Myxobolus of Bütschli. The polar capsules are equal in size as in the type species *Myxobolus mülleri* Bütschli of Europe which infests many fresh water fishes. Its inclusion in this genus emphasizes its relationship to *M. pfeifferi,* the cause of the devastating barbel disease, and to *M. cyprini,* which gives rise to the destructive fish-pox of the carp.

Only a few forms of the Myxosporidia have been reported within the limits of the United States. Gurley (1893; 111) listed nine species of which eight occur in fresh water hosts. A little earlier Linton (1891) had described a specially interesting form from fresh waters. The species infected was *Notropis megalops* and the locality from which they came was the Black River, Lorain County, Ohio. Since the host is a minnow closely related to that on which occurred the species described in this paper and since the localities are only a short distance apart on the south shore of Lake Erie, one is tempted to ask if the two parasites are not identical. A close examination of Linton's record and figures shows that they cannot possibly be the same species. Linton describes his form as producing globular or botryoidal masses on the side of the head and body and at the base of the fins. The illustration demonstrates clearly the distribution in groups or clusters and further the location of these masses on the body wall at the base of the fins. They occur in the specimen he figured at the base of the pectoral, ventral, anal, dorsal, and caudal fins, but in no case do they encroach on the membrane of the fin itself. They are confined exclusively to the surface of the body proper. The masses are made up of cysts that are distinctly confluent and in no case figured was one cyst discrete and separate from other cysts. The component cysts vary from two to three millimeters in diameter. Finally Linton describes the color of these cysts as white with minute patches of black pigment belonging to the skin of the host.

When these data are compared with those already given for the Put-in-Bay species the differences are conspicuous. The cysts of the latter species are usually single and even when grouped one can distinguish them as separate and entirely unconnected masses. They never form clusters or groups of a botryoidal character. In size they are only

one-third to one-half the dimensions of those in Linton's specimens. In general the cysts described by Linton are much more nearly spherical than those in the present species. Since Linton's material was not living when submitted to him it is uncertain what the appearance was in life, but in the letter from Mr. McCormick of Oberlin College that accompanied the specimens and described their capture no note is made of any color in the living material. It is certainly difficult to believe that any collector could overlook the very brilliant and striking color of the cysts of the Put-in-Bay species so that one may reasonably infer the absence of such coloring. The hosts are different species, one being a river form and the other a lake species, and the lake form was also much smaller than the other for which Linton records a length, exclusive of caudal fin, of 47 to 57 millimeters.

But the most striking difference is found in the location of the cysts. In the Put-in-Bay species they are always in the membranous expansion of the fins and never on the surface of the body, whereas, in Linton's species as already described the location is precisely the contrary. This difference in distribution is uniform and unvarying. No single exception is recorded for either species. The location of the cysts in Linton's species is not uncommon, although most forms that occur on the surface of the body are not confined so rigidly, as his figure indicates this form to be, to the region of the skin just at the base of the various paired and unpaired fins. But the new species described here is found only within the fin membrane, a most unusual location. The significance of this is discussed later, but the marked and constant difference in the location of the cysts may be regarded as clear evidence of the specific difference of the two parasites.

When the spores of the two forms are compared, one finds similar differences. They are, to be sure, much alike in general appearance and structure, but these features are merely those characteristic of all spores in this genus of Myxosporidia. If the drawings of Linton's spores are all of approximately the same magnification as is indicated in the explanation of his plate, then those spores vary in size far more than these. He states the dimensions of the spores in that species as 17μ long, 10μ broad, and 6μ thick, which makes them distinctly larger and different in proportions. They are more drawn out and show a concave taper wanting in the spore from the Put-in-Bay minnow. No comparison can be made of internal structure as he was unable to make out the polar capsules, threads, or nuclei in the spores. In view of all these features it is impossible to include the Put-in-Bay form in the species described by Linton.

The species of Myxobolus parasitize the gills, fins, scales, spleen, kidney, and muscles of the host. Commonly they are found in the

connective tissue of these organs and occur in several parts of the body. In our case the very specific localization of the parasite is distinctly noteworthy and in this the species differs from all others in the genus so far as is shown in literature available. The occurrence of Myxosporidian cysts in the fins of fishes is rare indeed. Minchin (1903: 339) cites only five cases: *Henneguya linearis* (Gurley) in *Ameiurus melas* at the base of the dorsal fin; *Myxobolus oviformis* Thél. in the fins, gills, kidney, and spleen of *Gobia gobio; Myxobolus mülleri* Bütschli from the fins and gills of *Leuciscus cephalus; Glugea acuta* Thél. from the connective tissue of the dorsal fin in *Nerophis aequoreus,* and from the same region in *Syngnathus acus.* From the same genus as our host species Minchin records only one case of infection and that in the skin of *Notropis megalops,* the case described by Linton (1891) and discussed elsewhere in this article. Careful examination of the literature shows that seven cases described as fin infection have been reported up to the present of which, except *Myxobolus seni,* all infect also other organs of the host. These species are as follows:

Myxobolus sp. Müller (1841: 480)
Myxobolus oviformis. Thélohan (1895: 351)
Myxobolus volgensis. Reuss (1906: 200-201)
Myxobolus gigas. Parisi (1912: 293-294)
Myxobolus seni. Southwell and Prashad (1918: 347)
Henneguya linearis var. Gurley (1893: 417)
Henneguya nüsslini. Schuberg and Schröder (1905: 56)

Of the four cases from the same region in the host, cited after Minchin, the last two concern marine fishes, and the first is doubtful. Of this case, Gurley (1893: 417) speaks as follows in the original description of the species: "In cysts *at the base* of the dorsal fin of *Ameiurus melas* Raf. from Storm Lake, Iowa, a spore occurs which I strongly suspect to be identical with this species, as it answers in every respect to the rather meager diagnosis." As the cyst is *below* and not *in* the fin, the location of the parasite does not at all correspond to that of our species. Gurley also in speaking of *M. linearis* (1893: 416) writes, "Cysts invariably* embedded in the subcutaneous tissue of some part of the head (especially the under surface of the lower jaw) of *Hybognathus nuchalis* Ag." Here again the location is not that of the species under consideration, as in the former case the cyst is really in the body near the base of the fin. Only one reference in the literature seems to agree in part with the description of *M. aureatus.* Southwell and Prashad (1918: 347) mention that cysts of *Myxobolus seni* were found only "on the median and caudal fins of *Labeo rohita,*" a species of fresh water fish taken in Mirpur, India. This is the only species of Myxobolus really and exclusively located *in* the fin. Unfortunately

*Among several hundred cysts one was seen *at the base* of the pectoral fin.

the authors have given only a very scanty description of this parasite. It is certainly different from the species described here and a detailed comparison is unnecessary.

Henneguya brachyura nov. spec.

Host: *Notropis anogenus.*
Location: in the cartilaginous fin ray.
Locality: near Put-in-Bay, Lake Erie.

In studying sections of the caudal fin of one of the minnows that was infected by *Myxobolus aureatus,* a species of *Henneguya* was found encysted in the fin ray. The cysts were rounded with slightly irregular contour and imbedded in the ray. In size they varied from 160μ in diameter up to 360 by 240μ. No particular cyst membrane could be recognized. The differentiation of the protoplasm into ectoplasm and endoplasm is distinct. The ectoplasm constitutes a layer 4 to 6μ thick, covering the entire surface of the parasite; it shows a

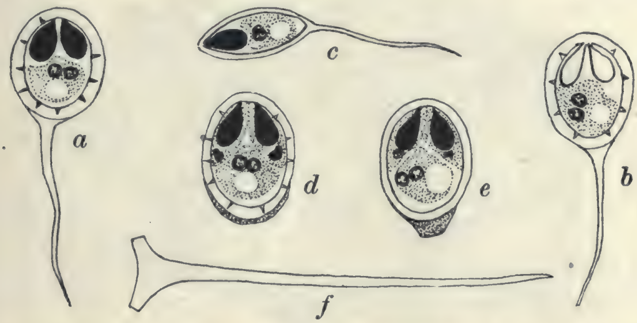

Fig. C.—*Henneguya brachyura* $\times$ 1,500, except *f*. *a, b, c,* different views of stained spores from section; *d, e,* young spores developing the tail; *f,* detached tail in a section, $\times$ 3,560.

very finely granular structure. The endoplasm is coarsely alveolar and filled with mature spores in the central portion, while numerous nuclei and young spores in various developmental stages are present in the peripheral portion.

The spore (Fig. *C*) is a rounded oval in front view but spindle-shaped with symmetrically built valves in profile. The shell is rather thick and the sutural ridge fairly well marked, the sutural edge exhibiting a variable number of folds (8 to 10). The pyriform polar capsules are usually of the same size and form. The tail is a single process, usually more or less bent or irregularly curved, very rarely being straight. In general it is sinuous with two or three shallow curves (Fig. *C, a*) and is rather short, tapering gradually to a point. In young spores which are less deeply stained by any stain, various developmental stages of the tail are easily recognized (Fig. *C, c, d*).

Giemsa solution stains the shell proper a clear blue, while the tail takes on a beautiful pink color, showing a distinct difference in affinity for dyes between the material in the tail and in the shell. According to Gurley (1894: 250), the tail of *Henneguya macrura,* with which the present species is closely related, was completely dissolved by concentrated sulphuric acid. It seems probable that the tail of this type is entirely different in its development from that of the ordinary bifurcated type, but further studies could not be made in this species owing to the small number of parasites available. In section, dimensions of the species are: length, 10 to 11.5μ; breadth, 8 to 8.75μ; thickness, 4 to 5μ; polar capsules, 3 to 4 by 2μ; length of the tail up to 17μ.

Among the known species of *Henneguya, H. macrura* Labbé (Gurley, 1894: 250) seems to be most closely related to the form under discussion. A comparison of two forms yields the following data:

	H. macrura	The Present Form
Habitat	Subcutaneous connective tissue Head of *Hybognathus nuchalis* Neches River, Texas, November, 1891	Fin ray of cadual fin; *Notropis anogenus;* Put-inBay, Ohio, August, 1898
Cyst	Large, elongated; size up to 6 by 2 mm.	Very small; invisible to naked eye; size in section, up to 360 by 240μ
Spore, similar features	Rounded oval; length 10 to 11μ, breadth 6 to 8μ, thickness 4μ, length of tail 30 to 40μ	Rounded oval; length 10 to 11.5, breadth 8 to 8.75μ; thickness 4 to 5μ; polar capsules 3 to 4 by 2μ; length of tail up to 17μ
Differences in the two spores	Sutural ridge without any folds; tail longer, slightly bent; polar capsules larger; valves very unequal	Sutural edge with distinct folds; tail shorter; sinuous; polar capsules smaller; valves usually equal

From this comparison it appears that in form and size the two spores are in close agreement, but the polar capsules differ very distinctly and the valves of the spore are rather sharply contrasted by their nearly equal form in the present type and their unequal form in the older species. Furthermore, the tail of the new form is only half as long as that in *H. macrura* and shows a wavy outline with two or three shallow curves instead of a simple, flat curve as in *H. macrura.* When one adds to these features which distinguish the two spores the radical difference in the size of the cysts, too great to be explained on the basis of differences in age and growth, it is hard to include both in the same species.

Finally Gurley emphasizes the location of the cysts, saying that in *H. macrura* the cysts are "almost invariably situated on some portion of the head," and stating that he had seen "but one exception, a cyst situated at the base of the pectoral fin," whereas the species under consideration was found actually at the opposite end of the body and

only in a cartilaginous ray of the caudal fin. I should not neglect to mention also the difference in hosts and the occurrence of the two parasites in separate geographic provinces. In connection with the morphologic evidence these facts are of significance in contrasting the two forms.

For these reasons I have decided that the new form cannot be brought under the older designation and propose for it the name *Henneguya brachyura.*

Henneguya salminicola nov. spec.

Host: *Oncorhynchus kisutch,* the silver salmon.
Location: connective tissue in body muscles.
Locality: taken in Stickeen River, S. E. Alaska.

In connection with studies I am carrying on with the Pacific salmon, the U. S. Bureau of Fisheries sent me preserved specimens labeled

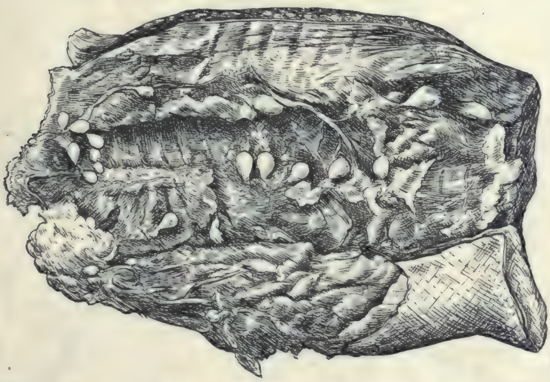

Fig. D.—Cysts of *Henneguya salminicola* in body muscles of Pacific salmon. Approximately half natural size. Preserved specimen.

"Pieces of Salmon with Cysts," collected by E. Lester Jones, Stickeen River, Alaska, about Sept. 10, 1914. Dr. Jones, who was at that time Deputy Commissioner and engaged in a trip to inspect conditions in Alaskan waters, received the fish within twelve hours of the time they were taken in gill nets so that they were in good condition. The saline solution in which they had been preserved was of a density of 5 or 6 per cent. and had kept the specimens passably well.

On examining the specimen (Fig. *D*) the observer was at once struck by the pale, whitish flesh around the cysts in clear contrast with the bright pink muscle usual in this fish. The zone of faded tissue surrounded the cysts to a width of 6 to 8 mm. The cysts themselves were pyriform, fairly uniform in size, and hard to the touch. They measured from 3 to 6 mm. in diameter. These cysts were especially conspicuous because some were pendant from the peritoneal wall, and

projected into the body cavity. They are not generally superficial in location as cysts appear everywhere through the muscle mass from the subperitoneal to the subdermal connective tissue, though of course all are subperitoneal in position. While they occur in groups in a certain sense, each cyst is entirely independent of those near it so far as can be determined by the unaided eye or by dissection. They certainly do not form botryoidal masses such as are found in some cases.

Sections demonstrate that the cysts are surrounded by a heavy capsule of connective tissue. Spores in various stages of development occur within the capsule and the mature spores are thickly massed together in the central area of the cyst.

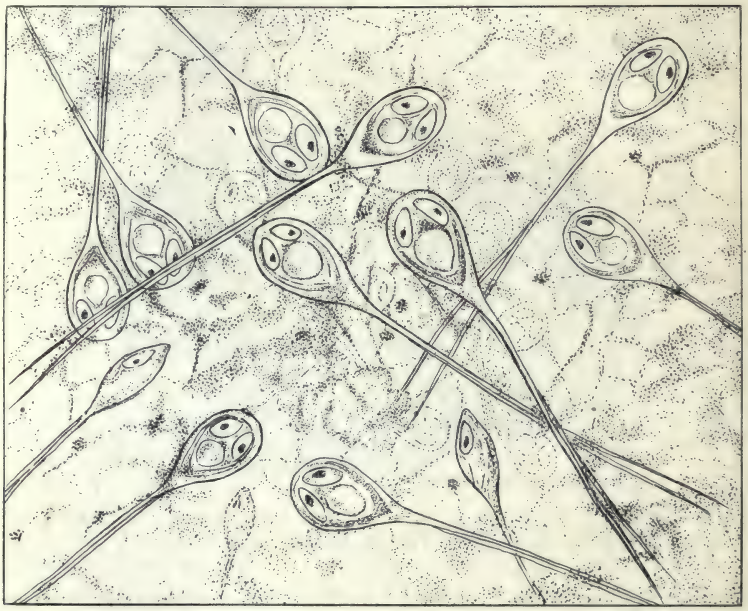

Fig. E.—Spores of *Henneguya salminicola* from section of cyst stained with iron hematoxylin. × 1,180.

There was, of course, no chance to study living material. The form of the spores is clearly shown in a drawing (Fig. *E*) made from a section of the cyst contents; the slide had been stained in iron hematoxylin and picric acid. The two long and delicate spines that project from the non-capsular end of the spore are in reality prolongations of the shell that are not pierced by the cavity of the spore. This form is characteristic of the genus Henneguya. Here the caudal spines are separate throughout their entire length but are roughly parallel and not divergent. The two nearly equal polar capsules are not contiguous along the median line but are separated by a band one-third to one-half the width of a capsule. A large iodinophilous vacuole, 3.4 to 4μ in

diameter, is conspicuous in the spore, but the polar filament coiled in the capsules cannot be distinctly seen in the preserved material.

A series of careful measurements was made of the spores and their processes. The body of the spore when measured in stained specimens "over all" varied in length from 11.97 to 14.25μ, on the average being 12.42μ, though the norm of length as calculated from the series was very close to 12μ. If measured to the base inside, stained specimens are 8.4 to 8.66μ. The width of the body of the spore varied from 7.12 to 8.43μ, with an average of 7.92μ and a norm of 8μ. The length of the tail was from 30.78 to 38.19μ, with an average of 34.54μ and a

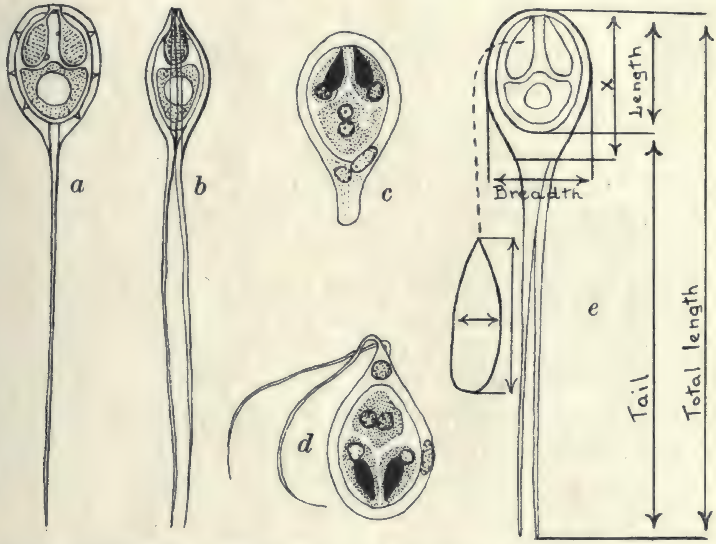

Fig. F.—*Henneguya salminicola; a, b,* unstained preserved spores; *c,* young spore; *d,* more advanced stage from smear stained with Giemsa; *a-d,* $\times$ 1,500; *e,* diagram showing limits observed in taking measurements, *x,* the length "over all," is not often used since the lower limit is not definitely marked.

norm of 35μ. In another set of thirteen specimens the average length was 12.44μ, the length of the tail 35.49μ; the average width of seven specimens was 7.63μ and the thickness of six specimens averaged 4.78μ.

The polar capsules range from 3.70 to 4.56μ in length by 1.59 to 2.85μ in breadth, or in the norm 4 by 1.8μ. One is almost always a little larger than the other, the difference being constantly about 0.25μ in length and half as much in width. Very few exceptions to this rule were met in a long series of measurements.

Since differences actually are found between measurements of spores of the same species in stained and unstained preparations, I give a table showing results obtained by two observers with different technic. The diagram (Fig. *F, g*) shows the limits used in making the measure-

ments recorded. A series of twenty-four to thirty mature spores was used in each case.

MEASUREMENTS OF SPORES OF HENNEGUYA SALMINICOLA

Measured by	Camera drawings	Ocular Micrometer	
Stained by	Iron hematoxylin	Giemsa	Unstained
Total length	42.75-52.44	43.5 -53.0	51 - 57
Length of spore body "Over all"	11.97-14.75	....	....
Length of spore body Inside base	....	8.4 - 8.66	8.6 - 9.5
Length of tail	30.78-38.19	34.25-36.75	....
Breadth of spore body	7.12- 8.43	7.5 - 8.5	8.6 - 9.5
Polar capsule Length	3.7 - 4.56	3.5 - 3.8	3.5 - 4.0
Polar capsule Breadth	1.59- 2.85	1.7 - 2.2	2.0 - 2.5

All measurements expressed in microns.

In form and size of spores this species resembles most closely *Henneguya zschokkei, H. schizura,* and *H. nüsslini. Henneguya nüsslini* was discovered in the trout by Schuberg and Schröder (1905), from whose description the following data are excerpted. The two cysts found lay in the subcutaneous connective tissue at the base of the dorsal fin. The spores were 12μ long by 8 to 9μ broad, and with the tail measured 32μ over all. The tail was split, but the two spines were never separated throughout their entire length. The polar capsules were 5μ long and 3μ wide; they do not meet along the median line, but are separated by a distinct space. The spore is rounded at the anterior end. In this respect and in the separation of the polar capsules the new species is like *H. nüsslini* and unlike the other species named above, but a comparison of the dimensions quoted shows that *H. nüsslini* has larger polar capsules and a larger spore body, whereas the total length is much less than in *H. salminicola.* These differences are too great to permit including the new form in the species *H. nüsslini.*

Henneguya schizura was first described by Johannes Müller but was not named until Gurley (1893: 417) called it *Myxobolus schizurus.* The parasite is found only in the orbit, encysted in the connective tissue of the eye muscles, in the sclerotic and between the latter and the choroid. It occurs in young *Esox lucius* and is present in May and June. Müller looked for it without success in specimens of the pike from North America. The two species agree in the length of the spore body (12μ), but in *H. schizura* the spore is only 6μ broad as against 8μ here, and the tail in the former is three to four times as long as the spore body, whereas here it is barely three times as long. The size of the spores is sufficient in fact to distinguish this form from the new species described here although the peculiar and restricted distribution of *H. schizura,* occurring only in the orbital tissue, precludes the possibility of considering the new species identical with it.

A close resemblance exists between the new species and *Henneguya zschokkei* which was first described by Zschokke and named by Gurley

(1893). It inhabits the subcutaneous and superficial connective tissue in the trunk muscles of *Coregonus fera.* The cysts are round or oval and of considerable size, up to 30 mm. in maximum. The spore body is 10μ long by 7μ broad. The tail is four to five times as long as the spore body and is composed of two slightly curved and diverging spines. In comparison with the species from the Pacific salmon, *H. zschokkei* has a smaller spore body and a longer tail. In the former species the tail filaments are nearly parallel and never divergent as in *H. zschokkei.* These points form an adequate basis for separating the two forms.

It is worthy of note that such parasites though common in many types of fish are almost entirely unknown in salmon of any sort. An examination of the literature shows only a single record of a Myxosporidian parasite in any European salmon. That is *Lentospora cerebralis* which is the cause of the gid disease (Drehkrankheit) of young salmon in the first year of life.

I have not been able to find a single record of the occurrence of Myxosporidia in an adult Europan salmon and not one in a salmon of any age from this continent. During the last fifteen years I have examined personally several thousand Pacific salmon of all species and have never seen one infected so far as could be detected with the unaided eye. In searching for diseased fish I have been aided by a large number of fishermen and other cannery employes who knew I was anxious to secure all such specimens and were desirous of aiding me so that the cases I have recorded represent those culled from several hundred thousand fish, and there is no entry in my notes of a Pacific salmon affected with any sort of myxosporidian disease.

It is hardy possible that this case could represent a seasonal disease which fell outside the time limits of my experience, for I have collected salmon in the Alaskan coastal waters at least as late as September 1, and the date of this find was only ten days later. Further, no report of such a condition has been transmitted to me by the many men in that region who have been interested in my work and anxious to participate in it.

Finally, one must consider the chance that this is a localized disease and infects only or chiefly the salmon that run in the Stickeen River. I have not collected or studied the Pacific salmon in that precise region and so cannot venture to pass judgment on the question. But if the infection is localized it must be held within narrower limits than are usually observed by the parasites of migratory or marine fish so far as I know them; for I have studied the salmon run both north and south of the Stickeen River and the channels connecting with it, and the fish boats which supplied the canneries at which I was working ranged nearly as far as the Stickeen; yet no fish were seen with a similar infection.

So far as I can ascertain, this is the first published record of the occurrence of a myxosporidian parasite in any fish from Alaskan waters. While the lack of records is very likely due in part to the limited attention paid to diseases of fish from that region, I am also inclined to believe, from my own observation, that myxosporidian parasites are rare in fish found in Alaskan coastal waters.

Papers Cited

Gurley, R. R. 1893.—On the Classification of Myxosporidia, a group of protozoan parasites infecting fishes. Bull. U. S. Fish Comm., 11 : 407-420.

1894.—The Myxosporidia, or Psorosperms of Fishes, and the epidemic produced by them. Rep. U. S. Fish Comm., 26 : 65-304.

Keysselitz, G. 1908.—Die Entwicklung von *Myxobolus pfeifferi.* Arch. Protist., 11 : 252-308.

Linton, E. 1891.—Notice of the Occurrence of Protozoan Parasites (Psorosperms) on Cyprinoid Fishes in Ohio. Bull. U. S. Fish Comm., 9 : 359-361.

Mercier, L. 1909.—Contribution à l'étude de la sexualité chez les Myxosporidies et chez les Microsporides. Mém. class. sc. Acad. roy. Belg., 2 : 3-30.

Parisi, B. 1912.—Primo contributo alla distribusione geografica dei missosporidi in Italia. Atti soc. ital. sc. nat., 50 : 283-290.

Reuss, H. 1906.—Neue Myxosporidien von Süsswasserfischen. Bull. Acad. Imp. Sc. St. Petersburg, 25 : 199-205.

Schuberg, A., and O. Schröder. 1906. Myxosporidien aus dem Nervensystem und der Haut der Bachforelle (*Myxobolus neurobius* n. sp. and *Henneguya nüsslini* n. sp.). Arch. Protist., 6 : 45-60.

Southwell, T., and B. Prashad. 1918.—On some Indian Myxosporidia. Rec. Ind. Mus., 15 : 344-348.

Thélohan, P. 1895.—Recherches sur les Myxosporidies. Bull. sci. France et Belg., 26 : 100-394.

EXPERIMENTS WITH STEAM DISINFECTORS IN DESTROYING LICE IN CLOTHING

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It has been shown by Nuttall (1918) that "the high standards of efficiency attained by regular disinfectors on the basis of their capability of dealing with bacteria or their heat-resisting spores do not appear necessary for mere louse destruction." This paper deals with the minimum requirements, as regards pressure, time and temperature, for louse destruction only. The question of sterilization does not enter.

The work was done at Camp Mills, L. I., N. Y., in response to a request from the Camp Surgeon to Dr. L. O. Howard, Chairman of the Subcommittee on Entomology, Medical Committee, National Research Council. The request was made at the suggestion of D. L. Van Dine, Captain, Sanitary Corps, who was in charge of the Sanitary Process Plants at Camp Mills, and the work was done in close cooperation with him and with Captain H. L. Gardiner, M. C., Assistant Officer in Charge, in an attempt to answer certain practical problems they had in mind. The principal question was whether the disinfecting process in use at these plants was effective in destroying lice in the clothing, and whether the process would still be effective if the time of treatment were shortened—thus increasing the daily capacity of the plants.

There are two Sanitary Process Plants in operation at Camp Mills. One, referred to as Plant No. 1, is built largely on plans and specifications from Major Harry Plotz (1919) of the Surgeon General's Office. The other, referred to as Plant No. 2, is "home made," so to speak. The nucleus of this plant was an old bath house, and the present establishment is the result of a process of addition and modification dictated by practical experience. Each plant consists essentially of

(1) Disrobing room. Here the men place blankets, overcoats and all other articles of clothing, except leather and rubber goods, in a barracks bag which is issued to them at the entrance. The bags are tied and tagged and placed in the carrier of the sterilizer.

(2) Bath; after a medical inspection the men pass into a bathroom where they are first painted with a kerosene emulsion, then pass to the showers. Here the amount of hot and cold water is not restricted, and the soap supply is liberal, and the men have time for a thorough bath. Those who, on inspection, are found infested with crab lice,

Phthirius pubis, are turned aside to a barber shop where axillary and pubic regions are shaved, after which they pass on into the bathroom.

(3) Drying and dressing room. Here the men dry themselves and are issued bath robes while waiting to receive their clothing from the sterilizers.

(4) Sterilizers. These are the essential factors in the destruction of lice in the clothing. At Plant No. 1 there is installed a large stationary sterilizer which measures about 18½ feet long and 5 feet in diameter. At Plant No. 2 there are twenty portable steam disinfectors arranged in two rows of ten each on opposite sides of a large shed. Each disinfector is rectangular and measures 30 by 42 by 84 inches.

The capacity of Plant No. 1 (February and March, 1919) was seventy men every 40 minutes; of Plant No. 2, 100 men every 40 minutes. The experiments reported below bear upon the question of the efficiency of these disinfectors.

SOURCE OF MATERIAL AND METHOD USED

The species dealt with is the body or clothing louse, *Pediculus humanus,* var. *corporis.* The stock lice were from material which had been reared through several generations during the preceding six months on a healthy individual. This stock was taken from Washington to Camp Mills by the writer. Volunteers were called for from the enlisted personnel of the Medical Detachment to act as hosts for the lice. Two men responded and were assigned by Captain Van Dine to the duty of feeding the lice and rendering other assistance in the experiments. The lice were kept in small pill boxes. These boxes are prepared by punching holes in both top and bottom and covering these openings with chiffon. Each box contained from 50 to 100 lice, together with a piece of cloth to which the lice cling, and to which they attach their eggs. For the purpose of feeding, these boxes were applied to the arm of the volunteers and held in place by means of elastic bands. This was done twice daily, and in the intervals between feedings, the boxes were kept in an electrically heated incubator at a constant temperature of 30° C. Every second day the lice were transferred to clean bits of cloth and returned to the boxes. The eggs or nits on the old piece of cloth, from which the lice had been removed, were counted, and each lot of eggs was put in a separate box. These were labeled and kept in the incubator. In the experiments these eggs were used as the test materials, on the assumption that any means which will destroy the nits will also destroy the active stages of lice, it being a matter of common experience that the nits are the more resistant. When the eggs were subjected to tests they were removed from the boxes and put in the pocket of a coat, blouse or shirt or inside a woolen

sock, thus approximating the conditions of natural infestation. In most cases a woolen sock was used, the bit of cloth with nits attached was put in the toe of the sock, and then a maximum registering thermometer was put in so that the bulb was near the eggs. The sock was then tagged and placed in the roll of clothing of some one of the men who were "going thru the mill" at the time.

The treatment to which the clothing is subjected consists of a preliminary vacuum, 10 inches, for 5 minutes; steam, 15 pounds for 15 minutes, reckoned from time steam is turned on; drying vacuum, 10 inches for 10 minutes.

Steam under 15 pounds pressure gives a theoretical temperature of about 250° F. (121° C.), and on several occasions maximum thermometers gave actual readings of 245° F. (118° C.). This temperature is developed only in the superficial layers of the bags as a rule. The temperature in the center of a mass of goods in a bag seldom goes so high, and then only in bags lightly and loosely packed and not subjected to pressure from weight of other bags above or alongside them. Whether a temperature sufficient to kill is developed in the center of a well packed bag depends on factors influencing the penetration of steam. Some of the factors are:

(1). The manner of packing the bags. The usual method was to spread out the three blankets, folded once lengthwise, on the floor, then the overcoat, blouse and other articles of clothing were arranged on top of these and the whole rolled up and put in the bag. There was, however, a great deal of variation in the way the roll was made up.

(2). The location of the bag in the load. It is obvious that those bags in the center with other bags pressed against them on all sides are more difficult to penetrate than those on the sides or top of the load.

(3). The size of the load. For every carrier there is a certain load beyond which more compression is necessary, which renders it most difficult for steam to penerate within the time limit.

(4) Treatment: Preliminary vacuum; number of inches. Steam under pressure; pounds and time.

(5). The nature of the goods treated, whether wool or cotton, closely or loosely woven.

(6). Moisture and temperature conditions of the goods when placed in the sterilizer.

EXPERIMENTAL DATA

The principal question as to whether the daily capacity of the plants could be increased by shortening the period of treatment of each lot was answered in the negative.

Experiment 1.—A load of twenty-five barracks bags filled with bath robes was given a very short treatment as follows: Preliminary vacuum, 5 minutes, 10 inches; steam, 5 minutes, 14 pounds reached; drying vacuum, 10 inches, 10 minutes. Lots of eggs and maximum thermometers were placed in center of two of the bags, one of which was located in the center of the load and one on top. In neither case were the eggs killed.

The two following experiments in which the period of exposure to steam was 10 minutes were done at the large sterilizer at Plant No. 1.

Experiment 2.—Bits of cloth with the nits attached were put in pockets of each of three wool blouses. A maximum registering thermometer was rolled up in each blouse in such a way that the bulb was in close proximity to the spot where the eggs were located. Each blouse was then put in center of a barracks bag filled with bath robes. These two bags were placed in the carrier with twenty-five other bags filled with bath robes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	165	5-7	34.5 C.	116	70.3
B	170	5-7	99.5 C.	0	0
C	158	3-5	77 C.	0	0
Control lot	75	5-7	30 C.*	53	70.6

* Incubator.

Location of Bags in Load.—Bag A was placed in the center of the load. Bags B and C were placed on top.

Treatment.—Preliminary vacuum, 10 inches, 5 minutes; steam, 15-18 pounds, 10 minutes (18 pounds reached at end of 10 minute period); drying vacuum, 10 inches, 10 minutes.

Experiment 3.—Twenty-five barracks bags were filled with bath robes, and in two of these the test materials were placed. The eggs were put in pockets of a wool blouse. A self-registering thermometer was rolled up in the blouse so that the bulb was near the eggs. The blouse roll was then rolled in four bath robes — thus approximating the conditions of a blouse rolled inside three blankets. The roll above described was put in a bag in which three bath robes had been stuffed in the bottom. The bag was then filled up with other bath robes and tied and tagged.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	137	2-4	58 C.	94	68.6
B	120	2-4	36 C.	81	67.5
Control lot	105	2-4	30 C.*	84	80.0

* Incubator.

Location of Bags in the Load.—Bag A was placed in the center of the load, with other bags below, above and stacked at the ends. Bag B was placed on top of the load.

Treatment.—Preliminary vacuum, 5 minutes, 10 inches (reached in 3 minutes); steam, 10 minutes, 15 pounds (reached in 3½ minutes, held 6½ minutes); drying vacuum, 10 minutes, 10 inches.

The peculiar case of a higher temperature recorded in the bag in the center of the load than in that one fully exposed on top, can only be explained on the assumption that some difference in the way the bags were packed interfered with penetration of the steam.

The results of these two trials with the 10 minute period for steam show three out of five cases in which eggs were not killed. Such a high percentage of failures demonstrated that the treatment was of too short duration.

A number of tests were made of the process in regular use at this camp, viz., a preliminary vacuum of 10 inches followed by 15 pounds steam pressure for 15 minutes (reckoned from time steam was turned in), and a drying vacuum of 10 inches for 10 minutes. The results indicated that this period could not be shortened; but on the other hand it was shown that the process was adequate provided certain precautions were observed. These have to do with conditions favoring thorough penetration of the steam, and will be discussed after first describing typical experiments in which all the conditions were favorable.

Experiment 6.—This test was run with the large sterilizer at Plant No. 1 in the course of regular operations and according to the regular procedure. Two wool socks were prepared by placing in each a thermometer and a bit of cloth with nits attached.

Location in Bags.—A was put in center of a roll consisting of three blankets, two O. D. shirts, two suits of underwear, one overcoat, one blouse, one pair breeches, four pair socks and one towel. B was put in center of a roll of three blankets, one overcoat, one blouse, one pair breeches, extra suit of underwear, wrap leggings and cap.

The underwear which the men were wearing was, after inspection, placed in the mouth of the bag on top the roll.

Location in Load.—Actual load was seventy-three bags, a normal load for this sterilizer. Bag A was placed near the center of the third row of bags, with one below and two above it. Bag B was placed on top of the load.

Treament.—Preliminary vacuum, 10 inches reached in 3 minutes; steam, 15 pounds, 15 minutes, 10 pounds reached in 6 minutes, 15 pounds reached in 8 minutes, held remaining 7 minutes; drying vacuum, 10 inches, reached in 5 minutes and held to end of 9 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	222	3-5	104.5 C.	0	0
B	310	3-5	93 C.	0	0
Control lot	125	3-5	30 C.*	83	66

* Incubator.

The two following experiments conducted at Plant No. 2 in the portable steam disinfectors also demonstrate the efficiency of this treatment when carried out properly.

Experiment 12.—Two wool socks, each containing a thermometer and cloth with nits were used as before.

Location in Bag.—Sock A was put inside a roll of three blankets. This was placed in the bottom of a barracks bag. On top of this roll the overcoat, breeches, sweater, socks and underwear were crowded. Sock B was put in a

roll of blankets in the center of the bag. Below the roll in the bottom of the bag was the overcoat, blouse and sweater. Above the roll in the top of the bag were underwear, breeches, shirt, socks, cap and wrap leggings.

Location in Load.—Normal load of ten bags was treated. Larger loads for this size sterilizer necessitate undue packing, resulting in difficulty of penetration. Bag A was placed in horizontal position near the center of the carrier. Bag B was put in a vertical positon at one end of the carrier.

Treatment.—Preliminary vacuum, 10 inches, reached in 1 minute; steam, 15 pounds, reached in 4 minutes, held 11 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 3 minutes, held 7 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	185	7-9	87.5 C.	0	0
B	162	7-9	118 C.	0	0
Control lot....	77	7-9	30 C.	58	75.3

Experiment 14.—This test was carried out in a portable disinfector in which a pressure of 15 pounds was attained. Two wool socks were used with thermometer and nits in each. A was put in a roll of three blankets, two sweaters, one shirt, one blouse. At the sides and top of this roll, after it was in the bag, were stuffed one shirt, two pair socks, two pair breeches, two suits underwear. Bag tightly packed. B was put in roll of three blankets, two suits underwear, two shirts, one sweater, one bouse, one pair breeches. Alongside and on top of this roll were stuffed two pair socks, wrap leggings, cap and underwear. The sterilizer was loaded with eleven bags, the two test bags being near the front end of the carrier.

Treatment.—Preliminary vacuum, 12 inches, reached in 3 minutes; steam, 15 pounds, reached in 5 minutes, held 10 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 5 minutes, held 5 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	128	6-8	94.5 C.	0	0
B	194	6-8	87 C.	0	0
Control lot....	163	6-8	30 C.	134	79.2

PRECAUTIONS TO BE OBSERVED

The following experimental data bring out some very practical points which have to be kept in mind if the process is to be efficient.

In the first place, less than 15 pounds pressure cannot be used with safety without lengthening the time of treatment. This was brought out in an experiment conducted at Plant No. 2 in a portable disinfector with safety valve adjusted so that only 12 pounds of steam was attained.

Experiment 11.—Two wool socks, each containing a thermometer and nits, were used as before.

Location in Bag.—The same as described for Experiment 12 above.

Location in Load.—There was a load of ten bags put in the sterilizer, and no one bag was completely surrounded by other bags, but had at least one side fully

exposed. One test bag (A) was in a horizontal position near the center of the carrier, and the other (B) was in a vertical position at the rear end of the carrier.

Treatment.—Preliminary vacuum, 10 inches, reached in 2 minutes; steam, 12 pounds, 12 pounds reached in 4 minutes, held 11 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 4 minutes, held 6 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	225	7-9	93 C.	0	0
B	287	7-9	51 C.	256	89.2
Control lot....	77	7-9	30 C.	58	75.3

Second, the sterilizers must not be overloaded. The tight packing necessary in an overload hinders penetration, especially in those bags in the center of the treated mass. This was shown in the following test:

Experiment 9.—This test was carried out at Plant No. 1 in the large stationary sterilizer during regular operations. Two wool socks were used as before, in each of which was placed a thermometer and a bit of cloth with eggs attached. Each was put in the roll of one of the enlisted men going through at the time.

Location in Bag.—A was put in center of a roll consisting of three blankets, one overcoat, one blouse, two O. D. wool shirts, one pair breeches, one extra suit underwear, one towel, four pair socks, one pair wrap leggings. B was put in another roll made up of practically the same amount of material except that there was one less O. D. shirt, and no towels, nor wrap leggings.

Location in Load.—A was placed in the center of the second row of bags in the carrier, with two bags below and two above it. B had the same position in the fifth row of bags near the other end of the carrier. The carrier was overloaded with eighty-nine bags, which were pressed together and tightly packed.

Treatment.—Preliminary vacuum, 10 inches, reached in 2 minutes, then steam was turned in; steam, 15 pounds, 10 pounds reached in 7 minutes, 15 pounds reached in 9½ minutes, held 5½ minutes, total 15 minutes; drying vacuum, 10 inches, reached in 6 minutes, held 4 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	185	5-7	41.5 C.	107	57.8
B	244	3-5	73 C.	0	0
Control lot....	118	3-5	30 C.	89	76.7

By way of comparison, the following test shows what a difference the location in a load makes.

Experiment 10.—This test was conducted in much the same manner as in the preceding one. Thermometers and nits were placed in socks and put in roll consisting of three blankets, one overcoat, one blouse, one pair breeches, one extra suit of underwear, socks, leggings and cap. The two rolls in this test were practically alike in amount of material and the method of packing.

Location in Carrier.—Both bags were placed on top the load, which was made up of ninety-three bags tightly packed in the carrier.

Treatment.—Preliminary vacuum, 10 inches, reached after 4 minutes; steam, 15 pounds, reached after 10 minutes, held 5 minutes, total 15 minutes; drying vacuum, 10 inches, reached after 6 minutes, held 4 minutes, total 10 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	310	3-5	105 C.	0	0
B	190	3-5	105 C.	0	0
Control lot	116	3-5	30 C.	89	76.7

The two experiments just described were carried out under practically the same conditions except for the location of the bags in the load. The amount of materials was the same in all. The bags were filled in the same way, and the treatment was the same. The difference in the location of the bags in the load had its effect on the results. These bags on top the load, and thus fully exposed, showed a good penetration, a temperature of over 105° C. being developed in the center of both. In those bags in the center of a heavy load (Expt. 9) penetration was rather poor. In one of the two the heat developed in the center of the bag was not sufficient to kill the nits.

A third point of importance to be borne in mind is the necessity of maintaining a full head of steam in the jacket of the sterilizer. With low pressure, the requisite 15 pounds pressure within is reached very slowly. This means failure to kill unless the time of exposure is lengthened, as in the following test.

Experiment 5.—This experiment was carried out at the large sterilizer at Sanitary Process Plant No. 1, in the course of regular operations. In each of two wool socks was placed a piece of cloth with nits attached and a self registering thermometer. Each sock was then placed in the roll of one of the enlisted men going through at the time. Each roll consisted of three blankets, one overcoat, blouse, breeches, two O. D. shirts, extra socks and underwear. The sock with the thermometer and eggs was rolled up in the center of this mass and the roll put in a barracks bag. The underwear worn by the men was, after inspection, placed on top of this roll in the mouth of the bag, which was then tied and tagged in the usual way.

The actual load in this test was ninety-three bags. Not all the bags, however, were completely filled, so that the carrier was not so much overloaded as the figures indicate.

Location of the Test Bags in the Carrier.—Bag A was placed on top of the load. Bag B was placed as near the center of the load as possible. It happened that the underwear worn by the man to whom Bag B belonged was infested with lice so that this offered a test on both the active and the egg stages of the lice.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	210	4-6	95.5 C.	0	0
B	140	2-4	104.5 C.	0	0
Control lot	155	4-6	30 C.	117	75.4

The lice in the infested underwear in Bag B were found to be dead.

Treatment.—Preliminary vacuum, 10 inches, reached in 3 minutes, held to end of 5 minutes; steam, 15 pounds, reached in 21 minutes, not held; drying vacuum, 10 inches, reached in 10 minutes, not held.

In the above experiment the steam pressure in the boilers was very low. Instead of establishing the required 15 pounds pressure in 7 or 8 minutes or less, and holding it for the remainder of a 15-minute-period, fully 21 minutes were consumed in reaching this pressure. The 15 pounds having been reached, it was released at once. The results are of interest therefore as indicating that penetration can be secured even when the steam pressure is low by a short extension of the time usually given for the application of the steam. It is assumed that the sterilizer is to be operated by one of sufficient intelligence to exercise good judgment in such circumstances. The failure to kill in one of the bags in the following test is to be explained on the ground that the pressure was rather low, requiring 9 minutes to reach the 15 pounds pressure.

Experiment 7.—Two wool socks, containing thermometer and nits, were used as in preceding experiments. Each was placed in the center of a roll of an enlisted man. The two rolls were practically alike as regards the amount of material. Each containing three blankets, one overcoat, one blouse, one pair breeches, extra suit underwear, one O. D. shirt, cap and wrap leggings. There was of course some difference in the way the rolls were made up, as no two men will make up a roll and fill their bags in exactly the same way.

Location in Load.—Actual load was seventy-three bags. Bag A was placed in second row with one bag below and two above. Bag B was placed in fifth row with two bags below and one above.

Treatment.—Preliminary vacuum, 10 inches, reached in 3 minutes; steam, 15 pounds, 15 minutes, 15 pounds reached in 9 minutes and held remainder of the period; drying vacuum, 10 inches, 10 minutes, 10 inches reached in 5 minutes and held 5 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	230	1-3	46.5 C.	217	94.3
B	292	1-3	96.5 C.	0	0
Control lot	122	1-3	30 C.	76	62.3

In Bag A all the contents of the bag was hot except for a very limited area near the center where the bulb of the thermometer happened to be located. In fact, the top part of the steel case protecting the thermometer was too hot to touch on removal while the bulb end was comparatively cool.

Fourth.—It is hardly necessary to point out the value of the preliminary vacuum in assisting the penetration of the steam. However, the following test is included as it brings out the point so clearly.

Experiment 8.—Three wool socks were used, each with a thermometer and a lot of eggs. Each was placed in the center of a roll consisting of three blankets, one overcoat, one blouse, one pair breeches, extra suit of underwear, two O. D. shirts, cap, socks, and leggings.

Location in Load.—Actual load was eighty bags. Bag A was placed in second row, two bags below and one above. Bag B was placed in fourth row, two bags below and one above. Bag C was placed in fifth row, two bags below and one above.

Treatment.—Steam, 15 pounds, 15 minutes, 10 pounds reached in 8 minutes, 15 pounds reached in 13 minutes, held at this point for 2 minutes; drying vacuum, 10 inches, 7 minutes, 10 inches reached in 5 minutes.

Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	168	5-7	45 C.	111	66
B	220	3-5	37 C.	190	56.3
C	298	3-5	104.5 C.	0	0
Control lot	105	3-5	30 C.	90	85.7

It will be noted that each of the test bags contained about the same amount of material and had the same relative positions in the load. No preliminary vacuum was produced, and failure of penetration is marked in two bags.

Fifth. — The differences in the way the rolls are made up have important influence on penetration. The following test gave results which show failure to kill in one heavy roll tightly packed as compared with success in a light, loose roll.

EXPERIMENT 13.—This test was also conducted with the large sterilizer at Plant No. 1. Two wool socks with thermometers and nits in each were put in roll of enlisted men's equipment as follows: A was placed in roll consisting of three blankets, one blouse, one breeches, one O. D. shirt, one cap, two pair socks. B was placed in a roll consisting of three blankets, one overcoat, one blouse, one breeches, two O. D. shirts, one extra suit underwear, four pair socks, one cap, one pair wrap leggings. It will be noted that B was a much heavier roll than A, containing one overcoat, one shirt, one suit underwear, two pair socks and one pair wrap leggings in excess of the material in A. Further, it was kept in compact condition by a web belt which the owner placed around it.

Location in Load.—A was placed in the third row of bags with one bag below and one above. B was placed in the fifth row with one bag below and one above it. The load was a normal one of sixty-nine bags, and the two test bags were placed so as to be about equally exposed.

Treatment.—Preliminary vacuum, 10 inches, reached in 3 minutes; steam, 15 pounds, reached in 11 minutes, held 4 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 5 minutes, held 5 minutes, total 10 minutes.

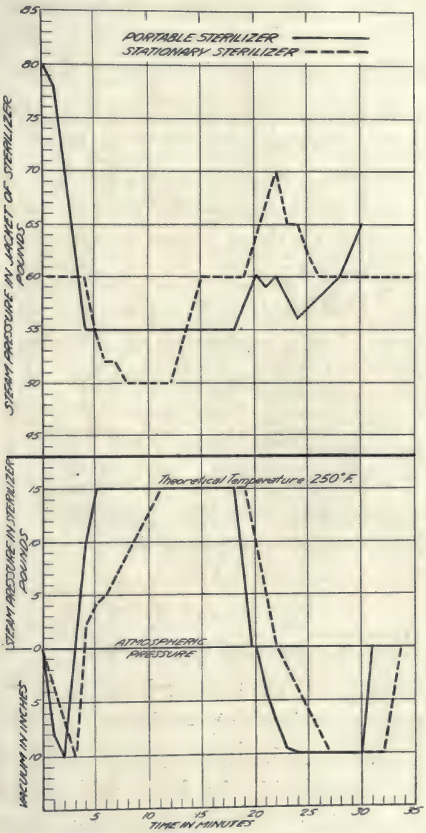
Results

Bag	Number of Eggs	Age of Eggs, Days	Maximum Temperature Recorded	Number Hatched	Percentage
A	210	5-7	85 C.	0	0
B	260	5-7	34 C.	244	94
Control lot	74	5-7	30 C.	46	62.1

In this experiment the difference between the two bags was in the amount of material they contained, and the fact that Roll B was kept tight by a belt. Location in the load and the treatment were the same. The results show that the penetration of the light roll (Bag A) was sufficient to kill all the nits, while in the heavy roll the heat failed to reach the center and none of the nits were killed. Bag B of course contains the usual amount of material which is ordinarily treated with success. The failure to kill was due to making the roll too tight.

A COMPARISON OF THE EFFICIENCY OF THE LARGE STATIONARY STERILIZER AND THE SMALL PORTABLE STEAM DISINFECTOR

A number of records were made of the operation of the sterilizers, noting minute by minute the pressure changes both in the sterilizers and in the jacket. Two of these records, selected because they show the usual changes when the sterilizers are operating well, have been charted (Fig. 1). It will be noted from this chart that the small portable sterilizers do the work more quickly than does the larger stationary



Comparison of the pressure changes in the large stationary and small portable disinfectors during normal operation.

one. The large sterilizer develops the 10 inch vacuum more slowly. It is also much slower in developing the 15 pounds steam pressure, and this is the most serious difference as regards practical results. Records show that the 15 pounds pressure is obtained in the portable sterilizer within 3 to 5 minutes from the time the steam is turned in. Reckoning the 15 minute period from the time the steam is turned on, the contents of the sterilizer is thus exposed to the 15 pounds pressure for 10 to 12 minutes.

In the large stationary sterilizer, the records show that 4 to 11 minutes are required to produce the 15 pounds pressure, thus leaving only 4 to 11 minutes for the application of the pressure.

In every test with the portable sterilizer, the 15 minute period of steam at pressure of 15 pounds was efficient in producing a killing temperature (75° C. and above) in the center of barracks bags packed in the usual way. In one roll, the goods were rolled very tightly, and the man strapped his belt around the roll and kept it tight. On removal from the sterilizer there was one cool spot in the roll under the thick padding of the shoulders of the overcoat. With this exception, which was caused by unusual conditions, we have never found failure of penetration when the portable sterilizers were working with 15 pounds pressure. A 12 pound pressure, however, is not always efficient.

In tests with the large stationary sterilizer, it was found that in three out of ten cases, killing temperatures were not produced in the center of the bags. All of these cases were bags in the center of a large load in the carriers.

It is the writer's opinion that the accompanying chart shows the probable explanation of this difference in efficiency. The solid line, representing operation of the portable sterilizer, shows that, after the 10 inch vacuum was produced, 15 pounds steam was developed within 3 minutes after the steam was turned on, and held for 12 minutes. The broken line, representing action of the large sterilizer, shows that 8 minutes were necessary to raise the pressure to 15 pounds, leaving only 7 minutes during which the goods were exposed.

SHRINKAGE TESTS

Two tests were made to determine the amount of shrinkage resulting from the sterilization process now in use. They were both done in one of the portable sterilizers at Sanitary Process Plant No. 2.

In the first test, two wool blouses, one new and one a re-issue, were folded up with a thermometer in the folds, and put into a barracks bag. This bag contained nothing but the blouses, and was placed on top of a load of eleven bags in the sterilizer. The object was to subject them to the full effect of the heat. They were subjected to the following treatment:

Preliminary vacuum, 10 inches, reached in 2 minutes; steam, 15 pounds, 15 pounds reached in 6 minutes, held 9 minutes, total 15 minutes; drying vaccum, 10 inches, reached in 3 minutes, held 7 minutes, total 10 minutes.

The temperature recorded within the folds was 246° F., which is within 4 degrees of the temperature theoretically developed at 15 pounds pressure.

The blouses were shaken out until cool and dry.

	Measurements	Before Treatment Inches	After Treatment Inches
A	Between shoulders	16½	16¾
	Length of back	29¾	29¾
	Right sleeve, inner seam	19	18¾
	Right sleeve, outer seam	25	24½
B	Between shoulders	16¼	16
	Length of back	29½	29½
	Right sleeve, inner seam	18½	18½
	Right sleeve, outer seam	25	25¼

In the second test, an overcoat, blouse and breeches were rolled up with a thermometer in the center and placed in a barracks bag. Another blouse and breeches were put in a second bag. These two bags were put in sterilizer without any other load. All the clothing in this test was now and pressed. They were treated as follows:

Preliminary vacuum, 10 inches, reached in 2½ minutes; steam, 15 pounds, reached in 2 minutes, held 13 minutes, total 15 minutes; drying vacuum, 10 inches, reached in 2 minutes, held 8 minutes, total 10 minutes.

The temperature recorded in the center of the larger roll was 220° F. It was doubtless higher at the outside of the roll, probably most of the goods were exposed to 245° F.

The clothing was shaken out and measured a second time after they were cool and dry, with following results.

	Measurements	Before Treatment Inches	After Treatment Inches
Overcoat.	Neck	19	18¾
	Length of back	41	40¾
	Right sleeve, outer seam	25	24¾
	Right sleeve, inner seam	19	18½
Blouse A.	Between shoulders	15	15
	Length of back	29¼	29
	Right sleeve, outer seam	25	25
	Right sleeve, inner seam	19¾	19½
Breeches B.	Waist	30	29½
	Left inner seam	26	26
	Left outer seam	38¾	38¼

The two following garments were folded loosely in another bag and probably were subjected throughout to a temperature of about 245 F.

Blouse C.	Between shoulders	15½	15½
	Length of back	28½	28¼
	Right sleeve, outer seam	24¾	24¼
	Right sleeve, inner seam	19¾	19
Breeches D.	Waist	31	31¼
	Left inner seam	26	26½
	Left outer seam	38¾	38½

The results show three measurements one-quarter inch longer after treatment; eight measurements remain the same, and fifteen measurements indicate slight shrinkage. The shrinkage averages only about one-tenth of one per cent. Allowance must be made for some variation in the manner of measuring, and also for the fact that the goods were not pressed after treatment before final measurements.

The above measurements are few in number. Nevertheless, it is safe to say that the sterilizing process as employed at this camp, causes very little if any shrinkage. There is no doubt, of course, that longer exposures to steam under pressure will cause more serious shrinkage. These results agree very well with those of Fulton and Staniford (1918).

CONCLUSIONS

If the penetration of steam is sufficient to produce a temperature of 75° C. (167° F.) in the center of a barracks bag (or other load of infected goods) all eggs and active stages of body lice will be destroyed. This conclusion, based on the above practical tests, agrees very well with laboratory experiments on fatal temperatures. Nuttall (1918) has shown that nits are killed in one minute at 70° C. in dry heat, and in 10 seconds at 70° C. moist heat.

If the disinfectors are operated efficiently on the time schedule now employed (viz., a 10 inch preliminary vacuum; 15 pounds steam pressure for 15 minutes, reckoned from the time the steam is turned on; followed by a 10 inch drying vacuum) the requisite temperature (75° C.) is attained in every case. By efficient operation is meant (1) the maintenance of a full head of steam so that the 15 pounds pressure in the disinfector is produced within 5 minutes, thus allowing at least 10 minutes for exposure; (2) overloading must be guarded against; (3) the individual bundles must not be rolled too tightly.

Little if any shrinkage of woolen goods is caused by this treatment. There is, of course, some wrinkling, but these wrinkles are not permanent but may be remedied by pressing.

The writer wishes to express his appreciation of the courtesy of the authorities of Camp Mills in permitting him to do this work in the camp, and especially to Lieut.-Col. James F. Edwards, Camp Surgeon, and Major Elmer Jackson, Assistant Camp Surgeon, for their active interest and help, and to the Surgeon-General's Office, War Department. for permission to publish the results.

Papers Cited

Fulton, D., and Staniford, K. J. 1918.—The Sterilization of Woolen Blankets and Uniforms. Jour. Am. Med. Assn., 71: 823.

Nuttall, G. H. F. 1918.—Combating Lousiness among Soldiers and Civilians. Parasitol., 10: 411-588.

Plotz, H. 1919.—The Importance of the Louse Problem. Jour. Am. Med. Assn., 72: 324-326.

TWO NEW PROTEOCEPHALIDAE *

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The known Proteocephalidae have been the subject of an adequate study by La Rue (1914). The present paper treats of two new species belonging to the genus Proteocephalus, collected by the writer from the Bitter Root Valley, Montana, in 1915 and 1916. The host of one species, *Ptychocheilus oregonensis* (Richardson), is a cyprinid, while that of the other, *Coregonus williamsoni* (Girard), is a salmonid. In addition to the fact that these parasites have certain unique characters that readily distinguish them from described species, is the record of new hosts for the genus and the location of these worms in a new geographical area.

Proteocephalus ptychocheilus nov. spec.

Host: *Ptychocheilus oregonensis* (Richardson).
Locality: Carlton, Montana.
Date of Collection: April 12, 1915.

Small to medium-sized cestodes, reaching a maximum length of 200 mm. and a maximum breadth of 1.45 mm. Scolex about 1 mm. broad, neck practically as broad. Median fifth sucker lacking; marginal four suckers about 333μ in outer diameter, deep. Proglottids distinct from region about 5 mm. distal to neck. Anterior proglottids two to three times as broad as long. Maturing proglottids longer than broad. Ripe proglottids almost twice as broad as long.

Genital pore lateral, slightly anterior to middle of proglottid, in a shallow depression, sometimes on one side, sometimes on the other. Cirrus pouch constricted in outermost third, dilated in inner two-thirds. Ductus ejaculatorius coiled somewhat in inner dilated region of cirrus pouch; surrounded by prostate glands. Vas deferens consisting of tightly coiled tubule, especially massed in mid-plane of proglottid, with coil extending distad two-thirds distance toward ootype. Testes about 60, irregularly distributed in more than one plane, each about 80 to 110μ in diameter. Vagina opening proximal to cirrus pouch. Vagina traversing vas deferens obliquely. Sphincter vaginae weak, if present. Receptaculum seminalis consisting of a slightly dilated proximal portion of vagina. Vitellaria composed of small follicles, closely crowded together in lateral field just inside longitudinal muscle layers. Ovaries irregular, sacculate. Uterus consisting of six pairs of lateral pouches and a median ventral pocket. Eggs with three membranes, 19.4 by 23μ in diameter of outer membrane.

The specimens on which this study was made consisted of four worms secured from the stomach of a single half-grown female squawfish or chappaul, *Ptychocheilus oregonensis* (Rich.), taken from the

* Contributions from the Zoological Laboratory of the University of Illinois, No. 146.

Bitter Root Valley, at Carlton, Montana, April 12, 1915. The worms were found free in the stomach along with a considerable amount of foodstuff. This host was infected also with *Holostomulum ptychocheilus* Faust.

The scolex of *Proteocephalus ptychocheilus* shows a blunt conical prominence with no indication of a fifth sucker. The four marginal suckers are superficially inconspicuous. Their general outline is spherical, while the long axis of the oval cup-like depression is at right angles to the surface of the cone (Fig. 1). The neck is hardly less broad than the head. Some little distance posterior to the head the worm becomes distinctly attenuated. In the region of maturing proglottids the segments are longer than broad (Fig. 2). Toward the distal end of the chain the proglottids assume a shape about one and two thirds as broad as long. In transection these proglottids are elongate oval.

The mature segments show all of the important sex organs. The genital pore is lateral, slightly proximal to the middle of the proglottid. It is situated in a slight depression; into it open cirrus pouch and vagina. The cirrus pouch is attenuate in its outer third. In this region the wiry cirrus organ is found. Internal to the band of the longitudinal muscles the cirrus pouch enlarges into a sacculate organ which is convexed ventrad. The ductus ejaculatorius works a sinuous course through the inner two-thirds of the contracted organ. The cirrus pouch as a whole occupies a region approximately one-third the width of the proglottid. It has a maximum width of 0.42 mm., and a diameter of 0.14 mm. From the region of the longitudinal muscle band to the innermost region of the pouch the ductus ejaculatorius is surrounded by a limited number of prostate glands. The vas deferens merges into the cirrus pouch imperceptibly. It is directed toward the middle field of the proglottid (Fig. 4), where a great mass of coils is found. Before the duct reaches the midpoint of the segment it turns distad and continues a sinuous course to a region somewhat proximal to the ootype. Here, in the distal third of the proglottid, it receives the vasa efferentia. The testes consist of about 60 oval bodies, located in more than one plane, mostly in the dorsal half of the worm (Fig. 4). They range in size from 80 to 110μ in diameter. Their connecting efferent ducts have not been observed.

The female organs have been observed as follows: The ovaries are a pair of glands in the distal portion of the proglottid, sacculate in outline, extending from the outer border of the vitellaria to the ootype. The organs are constricted internally so that a distinct T is formed with the oviduct junction. Soon after the oviduct emerges from the region of the ovary, it is surrounded by a definite sphincter (Fig. 5, *oc*);

usually designated as the oocapt. It measures about 38μ in cross section. From the oocapt the oviduct has a sinuous course. Toward the ootype, a little distance dorsal to this organ, it is joined by the vagina. The outermost limit of this organ is at the genital pore proximal to the cirrus pouch (Fig. 3). It runs inward, paralleling that organ until it reaches the mass of coils of the vas deferens. Here it crosses under the pouch obliquely toward the midplane, where it is directed distad, and, after a few cramped sinuous bends, runs directly toward the ootype. In this vicinity it joins the oviduct. There is no distinct receptaculum seminalis. Perhaps the dilated proximal region of the vagina, just before it enters the oviduct, functions as such an organ.

The vitellaria are small, numerous follicles, closely compacted into a pair of cords, each within the lateral curve of the longitudinal muscle area. In the region of the ovaries transverse collecting ducts of considerable size converge dorsal to the ootype and the common vitelline duct resulting courses ventrad to join the common oviduct-vagina before the latter runs into the ootype. The ootype is surrounded by a spherical mass of closely crowded gland cells, with a gross diameter of about 230μ.

The uterus emerges from the ootype as a tube of narrow bore (Fig. 5, *u*). After a considerable amount of coiling it runs proximad on the ventral side of the proglottid. It gives rise to six pairs of lateral pouches and a single ventral pouch, the latter in the plane of the cirrus sac (Fig. 4, *up*). This constitutes the original outlet through which the eggs gain access to the outside. The outermost membrane of the subspherical egg measures 19.4 by 23μ. The middle membrane has an average diameter of about 17μ. The primary membrane measures 13μ in diameter. The egg is filled with yolk material.

Proteocephalus ptychocheilus is most nearly related to *P. esocis* (Schneider). However, size differences, structure and shape of cirrus pouch and vas deferens, number and size of testes, amount of vitellaria and details of structure in the vicinity of the ootype, all serve to distinguish the present species as new.

Proteocephalus laruei nov. spec.

Host: *Coregonus williamsoni* (Girard).
Locality: Fort Missoula, Montana.
Dates of Collection: Oct. 25, 1915; Feb. 18, 1916.

Small to medium-sized Proteocephalids, reaching a length of 100 to 120 mm., and a maximum breadth of 1.62 mm. Head and neck not observed. Maturing proglottids longer than broad. Ripe proglottids about one and one-fourth times as broad as long. Segments distinct.

Genital pore lateral, proximal to middle of proglottid. Cirrus pouch elongate, biconvex, extending mesad one-third the width of the proglottid. Cirrus large, muscular. Ductus ejaculatorius bending back on itself at least once

within cirrus pouch. Vas deferens enlarges distad to enclose inner end of cirrus pouch. Coils of vas deferens comparatively few, in middle of the proglottid. Testes 40 to 50, in two planes, each testis 70 to 100μ in transsection. Vagina an attenuate tube proximal to cirrus pouch, describing a broad bow under the latter organ (Fig. 7) toward the ootype. Ovaries two, large, irregular. Vitellaria sparse, large, inside main longitudinal muscles. Uterus composed of nine lateral pouches; no ventral pouch observed. Eggs with three membranes, averaging 50 by 42μ in section.

The specimens from which this study was made were secured from two infections of *Coregonus williamsoni* (Girard), taken from the Bitter Root River at Fort Missoula, Mont., Oct. 25, 1915, and Feb. 8, 1916. In the first infection one specimen was found in the mid-intestine of the host. In the second infection five specimens were found attached to the wall of the intestine. In none of the specimens were the heads secured. The absence of the head and neck region makes the structure of the four suckers and the presence or absence of a fifth sucker a matter of conjecture. However, details of the mature and ripe proglottids identify this species as new.

The genital pore is marginal, irregularly alternating right and left. It comes almost to the surface of the marginal line, but at times shows a slight depression. The cirrus pouch is distal and slightly ventral to the vagina. The cirrus and ductus ejaculatorius are both short, so that the cirrus pouch extends mesad only about one fourth of the proglottid distance. The entire lumen is surrounded by a large number of closely grouped prostate glands. The inner end of the cirrus pouch projects into the enlarged outer portion of the vas deferens. It is reflexed acutely and reinforced by integument (Fig. 9). The vas deferens becomes slightly smaller as it courses toward the midfield of the proglottid. In this region it coils several times and here receives the vasa efferentia. The testes are distributed in two planes, mostly lateral to the uterine pockets. They number 40 to 50 and vary in cross section diameter from 70 to 100μ. The vasa efferentia have not been observed.

The vagina arises at the genital pore as a tube of small caliber. It runs mesad on the proximal side of the cirrus pouch and crosses under the vas deferens near the juncture of that organ with the cirrus pouch. It bends distad as it approaches the midfield and continues to the ootype (Fig. 7). The ovaries lie an appreciable distance from the distal margin of the proglottid. They are large irregular bodies, which may or may not become attenuated toward the midfield.

The vitelline follicles are large, sparsely scattered bodies, numbering about twenty on a side. They are often entirely lacking in sections of 8 to 10μ (Fig. 9). Their transverse collecting ducts unite to form a common vitelline duct which runs ventrad toward the ootype (Fig. 8).

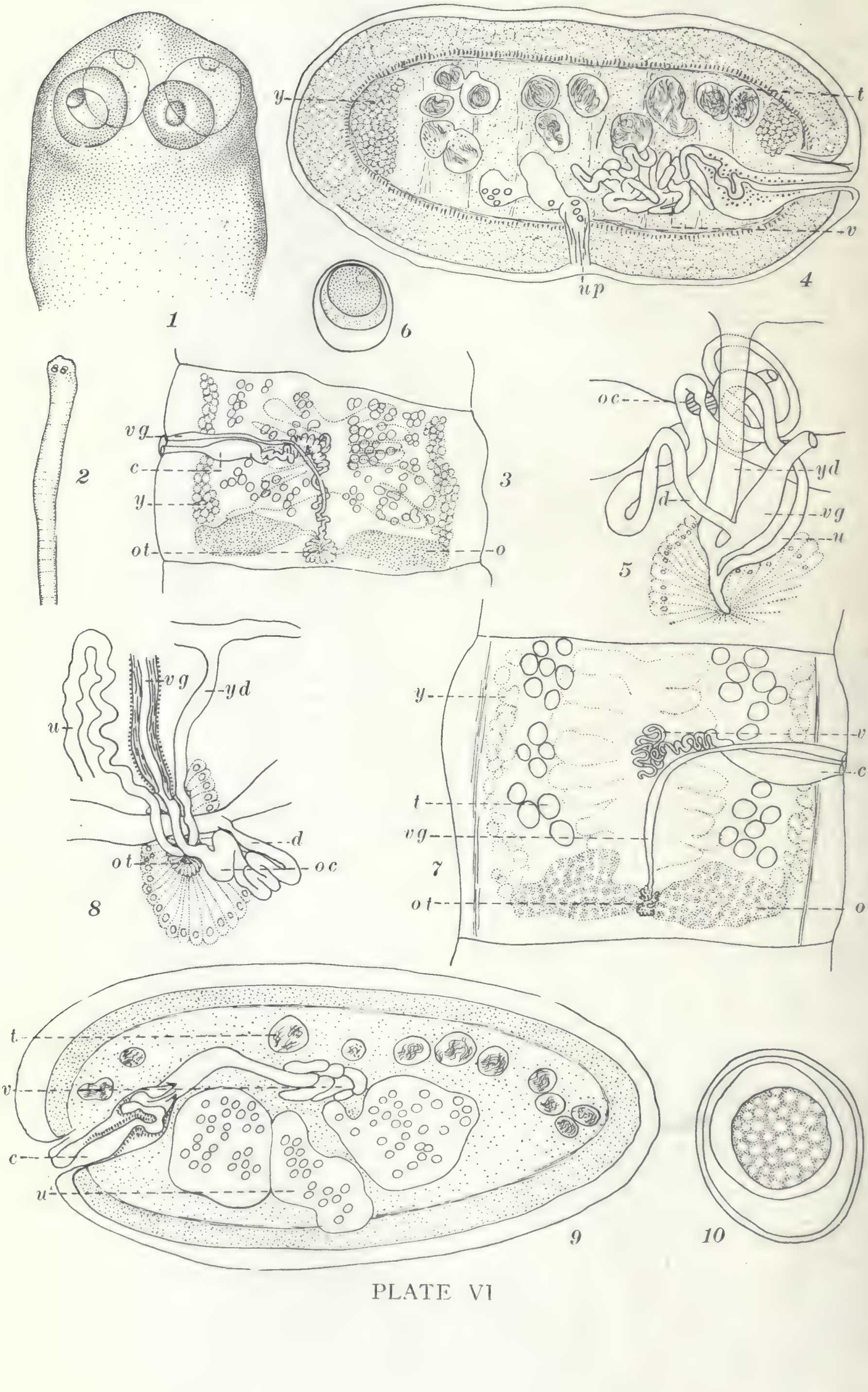

PLATE VI

The ootype with its surrounding glands is a pyriform organ. The gland cells consist of two types: small, densely crowded ones immediately surrounding the ootype, and larger ones enveloping the whole. From the common limb of the two ovaries arises the oviduct which coils on itself several times, and, after passing through a muscular enlargement, the oocapt (*oc*), is joined by the vagina. Thence it runs into the ootype. The proximal end of the vagina consists of a thick wall well supplied with inner longitudinal and outer transverse muscles (*vg*), surrounding a small lumen. Between the junction of the vagina and the oviduct and the ootype the vitelline duct merges into the common duct, so that the combined products are emptied into the ootype through one duct. The uterus emerges from the ootype as a small sinuous tube. Proceeding proximad it enlarges to form nine irregular pockets on each side of the midline. No ventral pocket for the emission of the eggs from the uterus has been found. The eggs are enveloped in three membranes, an outer one of a heavy consistency, with a diameter of 42 to 50μ, a middle one some 39μ in transection, and an innermost one of 25μ measurement (Fig. 10). The egg is filled with a dense mass of vitelline granules.

Proteocephalus laruei bears some resemblance to *P. cernuae* (Gmelin) La Rue (1914). It differs in the distribution of the testes, in the number of the vitellaria, in the details of the cirrus pouch and in organs around the ootype. In addition, the structure of the egg membranes is different.

Reference Cited

La Rue, G. R. 1914.—A Revision of the Cestode Family Proteocephalidae. Ill. Biol. Monogr., 1: 1-350, 16 pl.

Description of Plate

Proteocephalus ptychocheilus. 1. Enlarged view of head of worm, showing scolex, suckers and neck, × 34. 2. Habit sketch of anterior end, × 4. 3. View of mature proglottid, × 34. 4. Cross section of proglottid in region of cirrus pouch, × 54. 5. Diagram of organs in vicinity of ootype, × 105. 6. Egg, × 540.

Proteocephalus laruei. 7. Mature proglottid, × 34. 8. Detail of organs in region of ootype, × 105. 9. Cross section of proglottid in region of cirrus pouch, × 54. 10. Egg, × 540.

Abbreviations Used

c cirrus pouch.	*u* uterus
d oviduct	*v* vas deferens
o ovary	*vg* vagina
oc oocapt	*up* ventral uterus pocket
ot ootype	*y* vitellaria
t testis	*yd* vitelline duct

ON THE RESISTANCE TO DESICCATION OF THE INTERMEDIATE HOST OF SCHISTOSOMA JAPONICUM KATSURADA

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In an earlier paper (Cort, 1919: 489) I noted that specimens of *Blanfordia nosophora* (Robson), the intermediate host of the Japanese blood fluke, *Schistosoma japonicum,* which had been shipped from Japan to California in a dried condition, became active when placed in water. This resistance to drying is possible because *Blanfordia nosophora* is an operculate snail and is able to close the opening of its shell with its operculum. In this connection it seemed important to determine the degree of resistance to desiccation of this species of snail. The practical significance of definite data on this point is evident, since the destruction of the snail intermediate host is a promising line of attack in the control of any trematode parasite of man. Material for taking up of this problem became available when on June 23, 1919, I received through the kindness of Dr. S. Yoshida, of the Osaka Medical College, a large number of dried specimens of *Blanfordia nosophora* which had been collected in Japan May 28. Examination showed that over 85 per cent. of these snails were still alive, and that about 4 per cent. of the living snails were infected with the cercariae of *S. japonicum.*

The method of carrying on the experiments involved in this problem was as follows: The dried snails were carefully counted out in lots of one hundred and examined at intervals ranging from one to several days (Table 1). Each hundred snails at the time set for examination was placed in a beaker of water. After about fifteen minutes the snails would begin to emerge from their shells, and inside of two hours almost all of the living ones would be active. These snails were then crushed in watch glasses and examined carefully with a microscope for schistosome cercariae. The snails which had shown no signs of life after two or three hours were kept over night in the water. If any more proved to be alive they were also examined microscopically. In part of the examinations made during the first two weeks of the work, that is up to July (Table 2), the snails which showed no signs of life were also crushed and examined with a microscope. They were found without exception to be dead.

In my experiments the attempt was made to answer the following questions: First, how long can specimens of *Blanfordia nosophora* live in the dried condition? And second, do snails infected with the cercariae of *S. japonicum* show less resistance to desiccation than uninfected ones?

TABLE 1.—DATA SHOWING RESISTANCE TO DESICCATION OF BLANFORDIA NOSOPHORA

Date of Examination	Number of Days after Snails Had Been Dried	Number of Snails Examined	Number of Living Snails	Number of Living Snails Infected with Cercariae of S. japonicum
June 25	28	100	86	4
June 26	29	100	87	3
June 26	29	100	82	1
June 27	30	100	91	4
June 27	30	100	90	1
July 5	38	100	80	1
July 5	38	100	83	2
July 6	39	100	76	1
July 7	40	100	81	1
July 7	40	100	77	1
July 9	42	100	76	2
July 11	44	100	71	0
July 14	47	100	62	0
August 7	71	100	12	1
August 7	71	100	8	0
August 12	76	100	2	0
August 22	86	100	0	0
August 24	88	100	0	0

The data given in Table 1 shows that the length of life of *Blanfordia nosophora* in the dried conditions is comparatively short. From the fourth to the seventh week after the snails had been taken out of the water, June 25 to July 14, there was a distinct decrease in the number of living individuals. After ten weeks, August 7, only a small percentage of the snails were found to be alive, and finally in less than three months, August 22, all individuals were found to be dead. This last result was corroborated by data from two other entirely distinct batches of material of *Blanfordia nosophora*. A number of specimens were set aside from snails which were taken out of the water in Japan on March 27. On August 7, when this material was examined, all of the snails were found to be dead. Further material comprising about a pint of dried snails was brought from Japan by Dr. S. Yoshida. All were found to be dead when they were placed in water three months after they first became dry.

Table 2 contains seven one hundred lots taken from Table 1 in which microscopical examinations were made of the dead snails, as well as of the living. A large proportion of the dead snails were simply empty shells. Part of them, however, had apparently only recently died, and in some of these it was still possible to detect the infection with the cercariae of *S. japonicum*. Of the 700 snails included

in Table 2, 573 were found to be living and 127 dead. I have no record of how many of these 127 dead snails were merely empty shells. The table shows that 11 of the living snails, or 1.9 per cent., and that 7 of the dead snails, or 5.5 per cent., were infected with cercariae of *S. japonicum*. Since a large number of the dead snails were merely empty shells the proportion of infected snails among those which had died during the course of the experiment was much greater than 5.5 per cent. These figures certainly indicate a more rapid death rate among infected than uninfected snails.

TABLE 2.—DATA ON LOTS IN WHICH BOTH LIVING AND DEAD SNAILS WERE EXAMINED FOR INFECTION WITH THE CERCARIAE OF S. JAPONICUM

Date of Examination	Number of Days after Snails Had Been Dried	Number of Snails Examined	Number of Living Snails	Number of Living Snails Infected	Number of Dead Snails	Number of Dead Snails Infected
June 25	28	100	86	4	14	2
June 27	30	100	90	1	10	1
July 5	38	100	80	1	20	2
July 5	38	100	83	2	17	0
July 6	39	100	76	1	24	2
July 7	40	100	81	1	19	0
July 7	40	100	77	1	23	0

The data (Table 1) also show a distinct reduction in the percentage of infection in the living snails during the course of the experiment. In the first 500 snails examined, June 25 to June 27, the percentage of infection of living snails with the cercariae of *S. japonicum* was 3.44 per cent. In the second 500 snails examined eight days later, July 5 to July 7, the percentage of infection in living snails was only 1.51 per cent. On the other hand a striking case of resistance of an infected individual is shown in the first 100 snails examined on August 7 (Table 1), in which one infected individual was found out of 12 still living after the snails had been in the dried condition for ten weeks.

It was evident, however, that desiccation unfavorably affected the cercariae of *S. japonicum* within the snail host. All cercariae taken from snails which had been dry for a month or more were somewhat shrivelled and inactive. Many of these cercariae became plump and active after being in water for a while. Quite a number of cercariae, however, in each of these infected snails were permanently injured, and in some cases almost all the cercariae in a snail would be rendered entirely inactive. The conclusion can therefore be drawn in regard to the second question proposed, that not only are the infected snails less resistant to desiccation than the uninfected, but that the cercariae are injured and sometimes killed by a length of time in the dried condition that their host is able to resist.

It is perhaps significant to note in this connection certain habits of *Blanfordia nosophora* which are related to its resistance to drying. I have found it difficult to keep snails active in aquaria. They keep constantly climbing out of the water and drying on the glass. They became almost immediately active again when pushed back into the water. This habit of crawling out of the water seems to be related to unfavorableness of environment in the aquaria. In those aquaria in which there was present a considerable amount of food material, as indicated by a slight bacterial film on the surface of the water and in which the water was kept well aerated, very few of the snails crawled above the water. On the other hand, in aquaria containing little food material or in which the water was foul, almost all the living snails would soon be found dry above the surface of the water. It was possible to determine whether a given aquarium offered a favorable environment or not by the number of snails which remained active under the water. It is evident that this habit of going out of the water to escape unfavorable conditions would be of importance in maintaining the numbers of this species of snail in an environment such as the rice fields of Japan, where there are many changes in the level and the condition of the water. It would also make it difficult to destroy the snails in any given body of water, by the introduction of chemicals.

The data obtained from the experiments recorded in this paper have a definite relationship to measures for the control of Japanese schistosomiasis. The snail intermediate host has long been considered a vulnerable point for attack in the life cycle of digenetic trematodes injurious to man. In the fight against the sheep liver fluke, *Fasciola hepatica,* the killing of the snail intermediate host and the destroying of its breeding places by draining infected pastures, has always been the most effective measure of control. Leiper (1915) advocates this same method in the control of schistosomiasis in Egypt. He suggests that all temporary pools or ditches which harbor the intermediate hosts of *Schistosoma haematobium* and *Schistosoma mansoni* be drained. Since these snails are non-operculate and have little resistance to desiccation, Leiper's suggestion would seem to offer an effective method of control. The control of Japanese schistosomiasis in this way will prove more difficult on account of the resistance to drying of *Blanfordia nosophora.* The experiments outlined above show that this resistance is limited and wherever it would be possible to dry up breeding places for more than three months, the snails would be killed. Also even if the length of time of drying could not be carried to the full three months limit, some progress would be made on account of the reduction in numbers of the snails and the death of infected individuals.

CONCLUSIONS

1. The resistance to desiccation of *Blanfordia nosophora,* the intermediate host of the Japanese blood fluke, *Schistosoma japonicum,* is limited to about three months.

2. Desiccation unfavorably affects the cercariae within the snails and infected snails succumb more quickly than uninfected.

3. Individuals of *Blanfordia nosophora* will voluntarily leave the water and become dry under unfavorable conditions.

4. Measures for the control of Japanese schistosomiasis by draining the breeding places of *Blanfordia nosophora,* would be fully effective only if these places were kept dry at least three months.

Literature Cited

Cort, W. W., 1919.—The Cercaria of the Japanese blood fluke, *Schistosoma japonicum* Katsurada. Univ. Calif. Pub. Zool., 18: 485-507.

Leiper, R. T. 1915.—Report of the results of the Bilharzia Mission in Egypt, 1915, II, Prevention and eradication. Jour. Roy. Army Med. Corps, 25: 147-192.

A MOUSE OXYURID, *SYPHACIA OBVELATA,* AS A PARASITE OF MAN *

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In December, 1918, the late Dr. A. F. Coutant sent me from Zamboanga, Philippine Islands, a sample of fecal material containing tapeworm segments for identification. The sample was from an American Bohemian child living in Zamboanga. She was one of a family of five, all of whom were heavily infested by the worm in question.

Examination of the material showed the presence of eggs and of fragments of the rat tapeworm of man, *Hymenolepis murina* (*H. nana*). This species, until recently regarded as very rare in man, has been found in the course of the hookworm investigations in the South to be fairly common. Indeed, the prophecy made by Dr. Stiles soon after the commencement of that work, that "*Hymenolepis nana* will be found to be the commonest tapeworm in the United States" has been amply justified. Its minute size and the failure of physicians to make routine feces examinations had resulted in its being very largely overlooked, until the intensive studies of the hookworm campaign incidentally brought it to light. Of the more recent statistics there may be cited the studies of Frey (1915), who found this tapeworm in 32.6 per cent. of the inmates of the Texas State Orphans' Home. This percentage was exceeded only by that of hookworm infestation in the same group of 270 children.

While the tapeworm which had attracted attention thus proved to be one already noted in man, the search through the material led to the finding of eggs and two specimens of an Oxyurid hitherto unreported for man. Since the feces sample had been preserved by adding 6 to 10 per cent. formalin solution it is probable that the actual strength of the diluted solution did not exceed 3 to 4 per cent. Thus the fixation of the worms was imperfect, but careful comparison with specimens and with the detailed descriptions by Seurat (1916) have convinced me that the species under consideration is *Syphacia obvelata* (Fig. 1). This nematode has been until recently classed in the genus *Oxyuris,* but more critical work has resulted in its being separated by Seurat as the type of a new genus *Syphacia.* Like the tapeworms

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present in the same sample, it is a species which is known to occur in rats and mice.

DESCRIPTION

Both of the worms found in the sample were mature females. Unfortunately, one of the specimens was destroyed in laboratory class work before its value was appreciated. The following description, measurements and figure are from the remaining specimen mounted ventral side up in glycerin jelly.

Female (Fig. 2) elongate-fusiform, measuring 3.7 mm. in length by 0.3 maximum thickness. Cuticula finely cross-striate; two small cervical alae. Mouth surrounded by three broad lips. The body terminates in a long tail which measures from the anus to its tip 0.6 mm. About the anus are fragments of the sepia brown fungous growth noted by von Linstow and by Hall as common on the skin of many females of *Syphacia obvelata.*

The club-shaped oesophagus measures 300μ in length to the point where it terminates in a subspherical bulb. Bulb 100μ long. Vulva prominent, situated 100μ caudad of the oesophageal bulb, or 500μ from the anterior end. Excretory pore opening behind the oesophageal bulb, about 250μ in front of the vulva.

Eggs (Figs. 3, 4) are of the typical oxyurid type, asymmetrical, flattened on one side, measuring 125μ by 40μ. The embryo is evident in some of the eggs.

COMPARISON WITH OXYURIS VERMICULARIS

There is a striking difference in size between specimens of *Syphacia obvelata* and those of *Oxyuris vermicularis.* Our specimens of the former from man measure 3.7 mm. Other specimens of the same species from rodent hosts vary within moderate limits, Hall (1916) giving the range for females as 3.5 to 5.7 mm. On the other hand, females of *Oxyuris vermicularis* range from 9 to 12 mm. in length. The males of *Syphacia obvelata* measure from 1 to 1.6 mm. in length, as compared with 2 to 5 mm. for males of *Oxyuris vermicularis.*

Still more striking are the differences between the eggs of the two species, those of *Syphacia obvelata* (Figs. 3, 4) having over twice the length of those of *O. vermicularis* (Fig. 5), and also being more fusiform. The average measurements for those of *Syphacia obvelata* are 125μ by 40μ; for those of *O. vermicularis* 52μ by 24μ. Both are asymmetrical, those of *S. obvelata* being the more strikingly so.

More fundamental differences of structure have led various writers not only to distinguish generically between Syphacia and Oxyuris, but to remove the well-known human parasite *Oxyuris vermicularis* from

the genus Oxyuris. Seurat (1916) established for this species the genus Fusarella, but Railliet and Henry (1916) have shown that this must give way to the older name Enterobius Leach 1853. Thus the pin-worm of man, almost universally known in the medical literature as *Oxyuris vermicularis,* is more correctly designated *Enterobius vermicularis* (L. 1785) Leach 1853. The type of the genus *Oxyuris* is *Oxyuris equi* (Schrank 1788).

INFECTIONS OF MAN BY OXYURIS INCOGNITA

Shortly after this study was begun, there appeared a paper by Kofoid and White (1919) recording the finding of a nematode ovum, apparently undescribed, in 427 cases among approximately 140,000 soldiers examined at Camp Travis, Texas, and of various military units of the Southern Department (Texas, Oklahoma, New Mexico and Arizona).

In as far as there were published data the writers were certainly justified in stating that "this ovum is the largest ovum of intestinal worms encountered in human stools." Their measurements showed its average dimensions as 95μ by 40μ, with a ratio of length to diameter of 2.4:1. It is marked by the asymmetry typical of eggs of the Oxyuridae.

"The infected soldiers were examined for most part within two or three weeks after admission from civil life, hence the infection may be attributed to the region of their previous residence. The distribution has been determined on the basis of the place of enlistment of the infected soldiers. In 30,348 examinations made between July 28 and August 21, 1918, there were 361 cases of infection among troops in Camp Travis. These came from forty-eight states of the Union, with infections in twenty-two states."

Though no adult worms were discovered in these examinations, the writers concluded that the eggs are those of an Oxyuris to which they tentatively give the name *Oxyuris incognita.*

When Kofoid and White's report first came to my attention I thought it probable that we were dealing with the same parasite. This seemed the more possible, since they state that the egg which they found "is extraordinarily variable in size and proportions, its length ranging from 69 to 133 microns and its diameter from 33 to 43." On more careful examination, however, I found that none of the normal eggs from my specimens were as small as the 95μ which they give as the average. This was also true of eggs from available specimens of *Syphacia obvelata* from mice. Hall (1916) gives the length of eggs of this species as 110 to 142μ.

The other common Oxyurid infesting rats and mice in this country is *Oxyuris tetraptera.* The eggs of this species are much smaller than those of *S. obvelata,* averaging about 90μ in length by 36μ in width. It is possible that both of these species are capable of development in man, and that the wide variations in measurements obtained by Kofoid and White were due to mixed infections — a very common condition in the rodent hosts. It seems more probable that *Oxyuris incognita* represents a species as yet unknown except in the egg stage.

SOURCE OF HUMAN INFESTATION

From the available data relative to the case here reported, it is evident that the food of the child and of others of the family had been grossly contaminated by mice or rats. This accounts for the infestation by one of the commonest nematode parasites of these rodents.

Incidentally, it furnishes circumstantial evidence in favor of the view that *Hymenolepis nana* of man and *Hymenolepis murina* of rodents are one and the same species, as has been claimed, on morphological grounds, by various investigators. Grassi has shown, and we have repeatedly verified in experimental work, that *Hymenolepis murina* is able to complete its development in the intestines of a single host from eggs which have been ingested. The embryos develop in the villi of the intestines and the cysticercoids there produced drop into the lumen of the intestines and develop into the adult worms without the necessity of being transferred to another host.

Thus contamination of food by mice may be the cause of both cestode and nematode infestation of man. Why the nematode infestation is not more common is not clear. The failure to recognize it may be a question of the eggs being less abundant in the feces, or of being subject to marked seasonal variations in appearance, as suggested by Kofoid and White for the species with which they were dealing.

References Cited

Frey, J. H. 1915.—Helminthiasis at the Texas State Orphans' Home. Texas State Journal Med., 11:229-231.

Hall, M. C. 1916.—Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. Proc. U. S. Nat. Mus., 50:1-258.

Kofoid, C. A., and White, A. W. 1919.—A new nematode infection of man. Jour. Amer. Med. Assn., 72:567-569.

Railliet, A., and Henry, A. 1916.—Sur les Oxyurides. C. R. soc. biol. Paris, 79:113-115.

Seurat, L. G. 1916.—Sur les Oxyures des mammifères. C. R. soc. biol. Paris, 79:64-68.

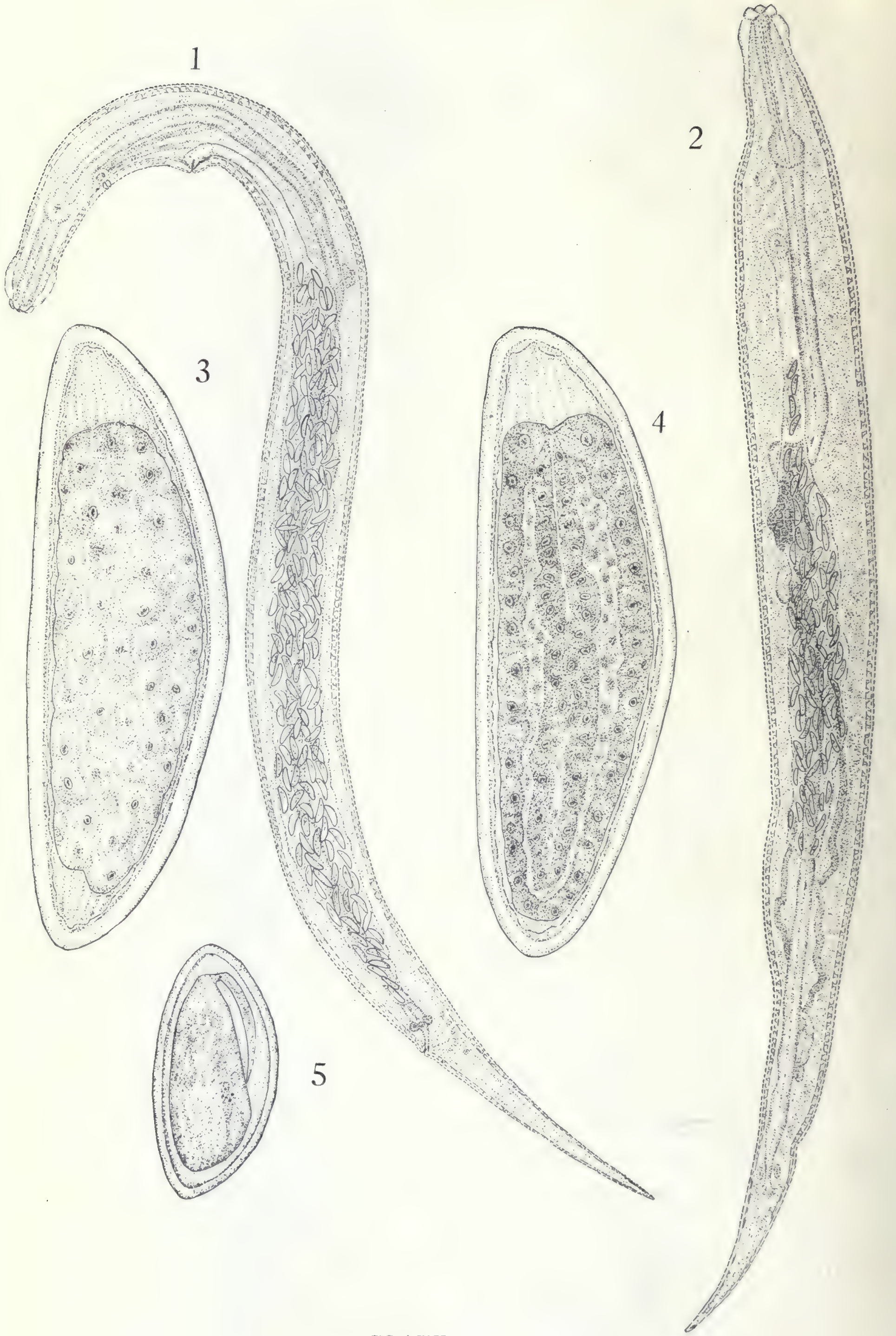

PLATE VII

EXPLANATION OF PLATE

Fig. 1.—*Syphacia obvelata* (Rudolphi 1802) Seurat 1916, from the cecum of the mouse, *Mus musculus*. $\times$ 50.

Fig. 2.—*Syphacia obvelata* from a child, Zamboanga, Philippine Islands. The preparation has been unduly compressed, and was twisted at the caudal end. $\times$ 50.

Figs. 3 and 4.—Eggs of *Syphacia obvelata*. $\times$ 720.

Fig. 5.—Egg of *Oxyuris vermicularis* drawn to the same scale as Figures 3 and 4.

The drawings were made under direction by G. H. Childs. Figure 5 was redrawn from Braun.

OBSERVATIONS ON *DIOCTOPHYME RENALE* IN DOGS

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In a series of routine autopsies on 3,200 dogs, a number of specimens of *Dioctophyme renale* (*Eustrongylus gigas*) were encountered. It seems of interest to tabulate the findings in such a large series of animals as regards the sex of the parasites, their length, the number of specimens encountered in a single host, the region in which they were located, the sex of the host, etc.

The autopsies were performed in the city of Washington between May 1 and Dec. 1, 1918, on dogs which came from the District of Columbia, Virginia, Maryland and Pennsylvania. The animals were of all ages, breeds and sizes.

At autopsy the thorax and abdominal cavity of each animal were opened and the viscera removed. In every instance in which a parasite was encountered the lesions were noted and the tissues sectioned.

In the following table the findings are briefly presented:

Sex of Host	Number of Parasites Found	Sex of Parasite	Length of Parasite	Location of Parasite	Condition of Kidneys
Dog 1, Male	One	Female	61 cm.	Peritoneal cavity	Right kidney atrophic and shrunken; imbedded in scar tissue. Left kidney normal
Dog 2, Female	One	Female	73 cm.	Peritoneal cavity	Normal
Dog 3, Female	One	Female	71 cm.	Peritoneal cavity	Normal
Dog 4, Male	One	Female	35 cm.	Peritoneal cavity	Normal
Dog 5, Female	One	Male	30 cm.	Peritoneal cavity	Normal
Dog 6, Female	One	Female	63 cm.	Peritoneal cavity	Normal
Dog 7, Male	Three	Male Female Female	25 cm. 61 cm. 63 cm.	Peritoneal cavity	Normal
Dog 8, Female	One	Female	38 cm.	Peritoneal cavity	Normal
Dog 9, Male	One	Male	28 cm.	Peritoneal cavity	Normal
Dog 10, Female	One	Female	51 cm.	Peritoneal cavity	Normal
Dog 11, Female	Two	Female Female	68 cm. 58 cm.	Peritoneal cavity	Right kidney atrophic and shrunken; imbedded in scar tissue. Left kidney hypertrophied
Dog 12, Male	One	Female	102 cm.	Peritoneal cavity	Normal

Twelve dogs in 3,200 harbored the parasite, a ratio of 1 : 266, or 0.37 per cent. It will be noted that five hosts were males and seven females. Two animals contained more than one worm. The majority of the invading organisms were females.

The organisms occurred in every instance free in the peritoneal cavity, and only twice could a portal of entry, through a partially destroyed kidney, be surmised.

The lesions of the peritoneal cavity are of interest. Constant trauma to the peritoneum, associated with the escape and absorption of blood, and the irritating effect of the organism's excreta and ova produce a chronic peritonitis. A dirty brown or greenish, odorless exudate of fibrinous character is observed in places clinging tenaciously to the roughened peritoneal surface. The omentum is often matted together with exudate and frequently adheres so intimately to the liver, spleen, pancreas and intestines that it is difficult to disentangle them. The mesenteric, preaortic and mediastinal lymph nodes are enlarged and deeply pigmented. Fibrous scars and adhesions are occasionally noted about the spleen and the liver.

On examining the thickened omentum or peritoneal surfaces microscopically, one finds them covered with a cellular exudate composed of polymorphonuclear leukocytes and mononuclear cells among which numerous ova are embedded. These ova are slowly disintegrating under the action of one or more giant cells by which each of them is surrounded. The cytoplasm of many of the mononuclear cells contains particles of detritus and pigment.

DISCUSSION

Interest attaches to this parasite because of its occasional occurrence in man. Blanchard (1886) reviewed the literature on the subject and found only nine human cases which he considered authentic. These had all been reported from Europe. Stiles (1898) states that up to that time no authentic human case had been reported in the United States. For an enumeration of the human cases and a description of the generic diagnosis, synonymy and other data concerning the parasite reference is made to Stiles. Stitt (1918) states that there seem to be seven authentic and nine doubtful cases of infection in man. The parasite in the human being is usually described as located in the dilated renal pelvis, and it is due to this circumstance that it is often spoken of as the giant kidney worm. In the fatal cases death results from peritonitis and hemorrhage following rupture of the distended kidney.

Balbiani (1870), in a number of experiments, attempted to transmit the infection directly by transferring the ova from one animal to another, but he was unsuccessful. From these experiments he concluded that an intermediate host must exist. He further observed the development of embryos from the ova and noted the fact that they would remain alive for many years in the presence of moisture. It is

thought that part of the life cycle of the organism is passed in fish, since larvae of the genus Dioctophyme have been found in certain species.

Besides in man, the organisms have been described in the dog, wolf, martin, mink, seal and other mammals. Geographically, they have an almost universal distribution.

In the United States and Canada this parasite has been most frequently described in the dog. Complete data on the length, sex of the parasite, etc., has seldom been given in the cases reported. Welch (1890) noted four of these organisms in the body cavity of dogs, and gave the length of one of them as 95 cm. Crowe (1907) mentioned two others, one of which occurred in the peritoneal cavity. He stated that the kidneys were normal and that there were no lesions of the peritoneum.

Riley (1916) found twenty-seven cases reported for the United States and Canada. He stated that in twelve of these the worms were found in the peritoneal cavity, but that in the majority they occurred in the dilated renal pelvis.

Hall (1917), besides reviewing the subject to date, added some observations of his own. His cases, added to Riley's, make a total of thirty-two for the United States and Canada. From some personal communications he later placed the total still higher. Hall stated that in one half of all the cases the organisms occurred in the peritoneal cavity. Stratton (1843) believed that the organisms invade the peritoneal cavity through the fallopian tubes, and Hall found that in nine out of ten cases in which the sex of the host was given, they were females. In my cases, however, only seven out of twelve of the hosts were females, showing that in all probability the fallopian tubes play no essential part in the entry of the parasite into the body cavity. That the parasite enters the peritoneal cavity through the kidney seems probable in those instances in which a ruptured or scarred kidney is associated with its presence. Frequently no lesions of the urinary tract are discoverable. In my series ten out of twelve hosts showed neither macroscopic nor microscopic lesions of the kidneys or ureters, and in these instances there is no clue as to how the organism gained access to the peritoneum. Crowe (1907) mentioned two cases, with the organism located in the peritoneal cavity, in which there were no discoverable lesions of the kidneys.

Sommer (1896), in examining fifty dogs in Washington for parasites, found this parasite in 2 per cent. Hall states that in examining a series of seventy-six dogs in Washington, he found none. In a series

of sixty-seven dogs in Michigan, he found it in two animals, or 3 per cent. The writer, in the present very much larger series, finds an incidence of only 0.37 per cent.

References Cited

Balbiani, G. 1874.—[Remarks] Compt. rend. soc. biol. Paris, 14: 125.

Blanchard, R. 1886.—Nouvelle observation de strongyle geant chez l'homme. Compt. rend. soc. biol. Paris, 3: 379.

Crowe, S. J. 1907.—The Parasites of Baltimore Dogs. Johns Hopkins Hosp. Bull., 18: 464.

Hall, M. C. 1917.—Parasites of the Dog in Michigan. Jour. Amer. Vet. Assoc., n.s. 4: 383.

Riley, W. A. 1916.—The Occurrence of the Giant Nematode *Dioctophyme renale* in the United States and Canada. Journ. Amer. Vet. Assoc., n.s. 2: 801.

Stiles, C. W. 1898.—Notes on Parasites. Med. Record, 53: 469.

Stitt, E. R. 1918.—Practical Bacteriology, Blood Work, and Animal Parasitology. Phila., Pa.

Welch, W. H. 1890.—Remarks and Exhibition of Animal Parasites. Johns Hopkins Hosp. Bull., 1: 72.

SARCOSPORIDIOSIS IN AN EAST INDIAN *

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Reports of cases of sarcosporidiosis of man are extremely rare. There are probably only two other undoubted cases in the literature: the case reported by Baraban and St. Remy in 1894, and the second reported by me in 1909.[1]

The finding of two cases is due probably to a rather persistent search for parasites in a very large postmortem service in the tropics. The infection is probably of little or no pathological importance in man, but as an example of what may be the lodgment of a sporozoon in a biological blind alley it has some interest.

CLINICAL HISTORY

Ali, a mohammedan Malabari, bullock cart driver and estate coolie, 30 years of age. Had come from Ponani, Malabar Coast, British India, two years before his death and had lived in Serembam and Ampang, Federated Malay States, for two years.

He was a patient of the District and General Hospitals, Kuala Lumpur, having been treated for malaria and hookworm infection. He was admitted to our ward[1] in the District Hospital on July 19, 1915, and was under treatment and care until September 28, the day of his death.

He was suffering from severe anemia of a type not uncommon in the Federated Malay States. Subtertian malarial plasmodia were found on admission by Dr. Barber, and he was treated for hookworm by Dr. Hacker and fifty-three hookworms were expelled. The erythrocyte count on the day of admission was 952,000.

His case was of interest to us, for he presented a picture of severe anemia which we believed was due chiefly to longstanding, insufficiently treated malaria. It is unnecessary to go into the details of his clinical course in the ward, for although extremely interesting as an example of untreated malarial cachexia it probably bears no relation to the slight infection by sarcosporidia which was found at the autopsy.

During his life his tongue presented a picture of desquamation and atrophy seen so commonly in the cachectic state following malaria

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that is sometimes called sprue. Monilia were detected in scrapings from the tongue, and his stools were frothy, but there was no invasion of the tissues of the gastro-intestinal tract by monilia. The patient's condition was apathetic, and he gave no evidences of pain or other symptoms referable to the musculatory system.

The postmortem disclosed the changes seen in severe anemia due to malaria, besides other lesions without special interest. The musculature presented no gross evidence of sarcocysts for these were too

Fig. 1.—Section of muscle, Case 2 "Ali," showing oblique section of muscle and a sarcocyst which through the obliquity of the section appears as two cysts.

small to be recognized by the naked eye. On inspecting the sections of tissues from the mouth, lips and tongue, sarcosporidia were encountered in one out of four blocks from the latter.

DESCRIPTION OF THE SARCOSPORIDIA

The section presented an elongated stippled body faintly encapsulated. The sarcocyst was not definitely imbedded in a muscle fiber, although lying parallel to the fibers near by and having a diameter or width two to three times that of the muscle fiber. The length was

two, three or four times its width, but there was some obliquity to the section thus shortening its real length.

The stippling was due to the nuclei of the sporoblasts which were slightly larger than those encountered in my first case, but much smaller than those of the sarcocyst of the sheep, hog, horse or rat, and on the whole were of the small type which I have described from the oppossum, hawk, guinea-pig and man (Darling, 1915). There was no evidence whatever of degeneration or inflammatory change, not even the slightest in the neighborhood of the sarcocysts.

Fig. 2.—Section of muscle, Case 1, J. H.; part (*A*) represents a piece of muscle taken July 2; part (*B*) represents a fragment removed July 13. The necrosis of the fibers and the cellular changes are due to typhoid fever and probably not to the presence of the sarcocyst.

DISCUSSION

Nothing is known of the man's habits previous to his admittance to the hospital. He had been an estate coolie and a bullock cart driver. He was also a mohammedan. From this we can state that like most East Indians he was practically a vegetarian, and that his diet consisted almost wholly of boiled rice, milk, some fruit and occasionally though rarely a bit of goat's flesh, chicken or fish. Meat could be almost entirely put out of his dietary. In our ward he got chicken, rice and

milk. There is no likelihood of his ever having tasted or eaten raw meat or fish. The source of the infection then is unknown.

Scott has published some interesting work on the epidemiology of sarcocyst infection in sheep and it is to be hoped that the natural mode of infection of this parasite will before long be cleared up.

Scott's recent paper (1918) on the seasonal incidence of sarcocyst infection in Wyoming is suggestive of the possibilities for the truth of my view that sarcosporidiosis as well as leishmaniasis and certain other infections are examples of parasitological blind alleys, and that sarcosporidiosis is very likely an infection by some sporozoon, very likely Neosporidia derived from insect or invertebrate through the contamination of food or drink, directly or indirectly, through droppings, and that the sporozoon after gaining the musculature of its strange host is unable to continue further its life cycle and escape from a compromising position.

Papers Cited

Baraban and St. Remy, 1894.—Sur un cas de tubes psorospermiques observés chez l'homme. Compt rend. soc. biol., Paris, (X), 1:201-202.

Darling, S. T., 1909.—Sarcosporidiosis with Report of a Case in Man. Arch. Int. Med., 3:183-192.

1915.—Sarcosporidia Encountered in Panama. Jour. Parasitol., 1:113-120.

Scott, J. W. 1918.—Notes and Experiments on *Sarcocystis tenella* Railliet. Jour. Parasitol., 5:45-60.

CONCENTRIC BODIES, PROBABLY OF PARASITIC ORIGIN, IN THE AUSTRALIAN SEA MULLET, *MUGIL DOBULA*

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The specimen, the subject of this note, was submitted to us by the Fisheries Department of the State of New South Wales in May, 1916.

The lesions consist of numerous small scattered areas, distributed through the musculature, composed of concentrically arranged cells, the larger areas showing degenerated centers. These pathological areas in appearance resemble somewhat the well-known cell-nests of a squamous epithelioma of man.

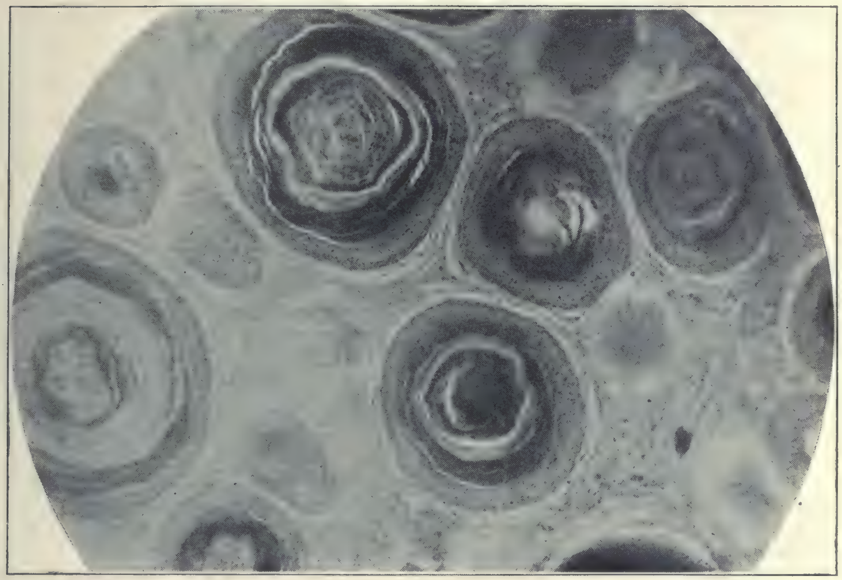
No parasitic bodies, protozoal or helminthic, could be recognized in the lesions, unless the cells composing the areas are themselves parasitic, which does not seem likely to be the case. Nevertheless, it is reasonable to assume that the lesions are due to the reaction to a parasite of some kind, past or present.

The notes are submitted with the object of calling attention to the condition and of elucidating, if possible, a satisfactory explanation of the appearances met with. The writer will be glad of any information in reference to the condition, which may perhaps be well known.

Description of the specimen.—Throughout the musculature are small, scattered, reddish, granular areas, the largest about the size of rice grains, many being smaller. These appear on the inner side of the ribs as small warty areas, and also extend on the back plate of the gills (all that remain of these, the fish having been cleaned for market). In places where the scales are thin, minute reddish spots, evidently due to the same condition, can be seen through them.

Microscopically, the affected tissue is found to be occupied by concentrically arranged masses of cells, the masses being usually spherical, but sometimes somewhat irregular. The smallest is about 60μ in diameter, whilst the largest reaches just over 1 mm. in diameter, the most frequent size being about 0.6 mm. The larger masses consist externally of layers of concentrically arranged elongated cells with medium-sized vesicular nuclei. The average diameter of these cells is about 8μ. These outer layers are succeeded internally by further layers of concentric cells, in which, probably from degeneration, the nuclei are condensed into small dark bodies about 2μ in size. In old lesions the center of the body stains deeply with iron hematoxylin. It is evidently much degenerated, the individual cells being mostly

indistinguishable, and is apparently somewhat calcified in part. Occasionally, in the center of the body are indefinite masses of black pigment. The smaller bodies represent presumably earlier lesions; some show the "outer" type of cell only. No central parasite could be recognized in either the early or late lesions. The lesions are found amongst the muscle tissues in the deeper parts, and even in the subcutaneous tissues they are seen in proximity to muscular masses.



The accompanying photograph illustrates the appearances presented better than a description in words.

NOTES

The Molteno Institute for Research in Parasitology has been founded at the University of Cambridge (England) by a gift of $150,000 for building and equipping the laboratory and for providing an income to maintain it. The generous gift comes from Mr. and Mrs. P. A. Molteno and is in response to an appeal showing the need of such an establishment which was prepared by Dr. G. H. F. Nuttall, well known for his work in parasitology and for his editorship of the English publication bearing that name. This appears to be the first institute established specifically for this purpose.

The genus Demodex, monographed by Stanley Hirst, is the title of the first number of Studies on Acari, published by the British Museum (London). This work presents a wealth of new and important data on the structure, biology and classification of a group hitherto little known.

An investigation of the Louse Problem by William Moore and A. D. Hirschfelder which appeared recently in Research Publications of the University of Minnesota, deals with methods of rearing, controlling and destroying lice, and pathological effects of the bite of the clothes louse. It is an exhaustive and critical study that deserves to be widely known.

PLATE VIII

EXPLANATION OF PLATE VIII

Fig. A. The mature sporocyst of *Leucochloridium problematicum.*

Fig. B. The mature sporocyst of *Leucochloridium macrostomum,* after Carus.

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LEUCOCHLORIDIUM PROBLEMATICUM N. SP.*

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While examining snails at Fairport, Iowa, in the study of *Lissorchis fairporti* Magath, during the summer of 1917, two snails were found which harbored mature sporocysts of a parasitic trematode of the genus Leucochloridium, and which is to be designated as *Leucochloridium problematicum* n. sp.

The first snail was obtained from Experimental Pond 2 D and was a species of *Succinea retusa* Lea, while the second was a *Planorbis trivolvis* Say, from Experimental Pond 5 D. On further study seven other snails, all of the latter species, were found to be infected with younger sporocysts which could be demonstrated by careful dissection and examination of the livers. In all, records of 209 snails were made, including the following species and numbers of each:

Planorbis trivolvis	120
Succinea retusa	48
Lymnaea obtussa exigua	23
Physa heterostropha	18

This gives an infection of 4.5 per cent. for all; or 2 per cent. for *Succinea retusa* and 7 per cent. for *Planorbis trivolvis*.

In examining the literature for reports of Leucochloridium from America, only two references can be found, and both of them are open to question. The first is that of Call (1898), who remarks under the description of *Succinea obliqua* in Indiana, "the tentacles are rather large and thick, club-shaped, and are often the home of a stage in the development of a planarian."

The second account is given by Ward (1917), who states that Mr. Bryant Walker, in a personal letter, reported finding the larval stage of a Leucochloridium in *Succinea ovalis* in Michigan. It is of course impossible to say whether or not these reports refer to the species or even the genus herein described, but it is possible that since both reports come from nearby states the same worm was found.

* Contribution from the Laboratory of the United States Bureau of Fisheries, Fairport, Iowa.

Figures 3, 4 and 5 were drawn by Mr. C. W. Shepard, artist in the Department of Anatomy, University of Illinois, College of Medicine, Chicago, Ill.

Leucochloridium is a genus created by Carus in 1835 to contain a species of larval trematode which he named *Leucochloridium paradoxum.* However, Zeller (1874) showed that this larval form developed into the adult trematode that Rudolphi found in the cloaca of *Motacillae lusciniae* in 1803. This early parasitologist called the worm *Fasciola macrostoma,* but in 1809, recognizing its difference from the liver fluke of the sheep, changed the genus name to Distomum, designating the worm as *D. macrostomum.* Monticelli (1888) thought that the description of *D. macrostomum* was sufficient to justify creating a new genus to contain it and called the genus Urogonimus naming *U. macrostomus* as the type. He gave practically no description of the genus except to call attention to the fact that the genital pore was posterior. In 1893 he described *U. ceratus* as a new species. Braun (1896) used in his text both Leucochloridium and Distomum in referring to the species of Carus, but apparently accepted the work of Monticelli, and the genus Urogonimus. Looss (1899) also accepted the genus Urogonimus and described a new species, which he called *U. insignis.* At this time he established a new genus to contain *Urogonimus rossittensis* (Mühling). The two genera were then placed in the subfamily Urogoniminae, with Urogonimus as the type genus.

Stiles and Hassall (1898) stated that they were inclined to adopt *Urogonimus* as a genus in view of the fact that the law of priority did not entitle larval genera to be used as names for adults.

It remained for Poche (1907) to make this final step, and he stated that the genus should be known as Leucochloridium, with *L. macrostomum* (Rud.) as the type, which necessitated the substitution of Leucochloridiinae for Urogoniminae of Looss. This of course does away with the original specific name of Carus for the larval form.

The decision of Poche has been accepted by Lühe (1909) who places the genus in the superfamily Distomata Retizus as Group IV, forms with genital opening posteriorly, and gives a clear cut generic description. He states that the only species found in Germany is *L. macrostomum,* the adult of which has been found in *Rallus aquaticus L., Gallinula chloropus* (L.), and *Ortygometra porzana* (L.). The larval form he reports from *Succinea putris* L. Ward (1917) also accepts this disposition of the genus, and it seems to the author to be the best that can be made, since the genus was clearly defined under the name Leucochloridium and could be so recognized.

Apparently the only larval form that has been reported is that of Carus, who first found it in *Succinea amphibia* in 1833 (reported in 1835) on an island in the Elbe. He states that a friend of his found a similar sporocyst in a snail in Halle in 1825, and it has been reported from Leipzig by Heckert. The description of Carus is of course, not complete, and two of his figures are reproduced in the present paper.

He found a snail whose tentacles seemed to be stained green and white; on close study these were seen to be two bodies, not unlike the larvae of certain insects, which could be retracted into the body of the snail or extended into the tentacles. The mass pulsated and was white with green bands all round it, and brown "warts" at the tip. Near the tip several bands were wider than the others. At the proximal end of the green and white body was a thread-like structure which he later traced into the liver of the snail, where were found numerous small knob-like bodies connected to it by other threads. He tore one of the larger sacs open and found it contained about 300 small worms, each in a little sac of its own. On the inside of the sporocyst were little bodies or cells which he said developed into the worms. These were, according to him, one-sixth of a line long and had well developed organs, which he did not describe in a very complete manner. He further called attention to the fact that the individual sacs of the worms had connections with the two suckers. In discussion of his findings he quotes the following interesting note from Rudolphi (1809): "in tentaculis *Helicis putris L.* Augustus Ahrens Halae Septembri corpuscula reperit, quae omnino huc facere et novum genus sistere videntur."

Following this many observations were made upon this worm, by Leuckart, von Siebold and others, until finally Zeller (1874) showed the relation between the larval form in the snail and the adult worm in the cloaca of singing birds, found many years before by Rudolphi.

What Zeller described in brief Heckert (1889) enlarged upon and gave a very extended account of the histology, morphology and life history of the species. He found the larvae not rarely in the snails near Leipzig. The ramifying tubules penetrated the liver of the host; these tubules were filled with a serous fluid and germ-spheres from which the larvae developed. Parts of the tubules extend up into the tentacles, and these portions are highly colored. The tubules have three muscular layers, a longitudinal, a circular and a diagonal layer. Below this dermo-muscular layer is bright green pigment arranged circularly. The sporocyst has the same structure as the tubules. There is an exterior cuticula, a dermo-muscular tube, and inside this a layer of cells which vary in size with the stage of development. Still inside of this is a membrane with distinct cellular elements, the cells of which differentiate and drop off, eventually forming the larvae. The order of development of the organs of the larvae is as follows: genital cells, suckers, pharynx, enteron, excretory system and nervous system.

There is a double ecdysis, but the cuticula remains with the larva, forming a protective covering, with fluid between it and the worm and the suckers connected to the covering. The Sylviidae are the true hosts and the adult is found in the cloaca; eggs are formed at the end

of eight days. The shells are thick, $\frac{1}{30}$ mm. long and at the hinder end of a ciliated comb, there is a powerful cone which acts as a steering organ. When the eggs were fed to Succinea after eight days they were found in the liver; they bored through the stomach wall with their head cones. Heckert further noted that in the adult the genital pore is terminal or dorsal and not ventral.

Zeller (1874) says the larvae are white, the body flat and broader at the anterior end than posteriorly. The integument is thickly covered with fine hairs or cilia. The anterior end of the body is very peculiarly constructed with its collar-like projection of the entire integument, which overlaps the mouth sucker and sticks up high behind. There are great numbers of unicellular glands in this projection. The suckers are large and very powerful, the mouth sucker being larger than the ventral one. The digestive apparatus consists of a powerful pharynx, a very short esophagus and the intestinal crura which describe a graceful arc as they extend to the posterior part of the body. The excretory system has a short contractile terminal bladder. The rest of the system consists of small tubules which seem to end blindly as fine branches.

The genital organs are quite well developed, and both the male and the female organs lie in the posterior part of the body. The two testes are more or less egg-shaped. One lies immediately posterior to the ventral sucker on the right, the other further back and on the left side of the body. The duct from the first leads out towards the other testis. Both ducts join together and the vas deferens passes posteriorly, lying in the posterior part of the body in a spherical cirrus sac, ending in an extensible cirrus at the posterior end of the body. The cirrus is not easily demonstrated in the larvae, although it is prominent in the adult.

The ovary lies between the two testes, nearer the posterior one. It is round in shape and has a short duct leading from it. This receives first the duct by which the spermatozoa enter from the vagina which opens on the dorsal surface anterior to the excretory opening. After this the duct bends and receives from beneath, at right angles, the ducts from the yolk glands. In the larvae these ducts enter the oviduct in a "rounded-body" which was thought to be the shell gland. The oviduct continues as the true uterus, which at first passes to the left and anteriorly to nearly the pharynx, there crosses to the right above the ventral sucker and descends to the posterior part of the body on the right side where it opens near that of the cirrus sac. The uterus is empty in the larvae, but in the adult the eggs are dark brown and measure 0.025 mm. by 0.014 mm.

The description of the adult *L. macrostonum* does not concern us here, but one might state that the adult structure is accurately fore-

casted by the larvae, and that in the ceca of the singing birds they reach a maximum length of 1.8 mm. by 0.80 mm.

However, the description of *L. insignis* is important for us, and following is given in brief Looss' (1899) description of the species. The worms were found in the cloaca of *Fulica atra*. They are about 3 mm. long and 1.35 mm. broad. The anterior end is thicker and more rounded, the posterior flattened and also somewhat rounded. The suckers are very powerful and large; the mouth sucker has an elevated margin around it. This sucker (0.73 mm.) is as large as, and perhaps larger than, the ventral one. The skin is not ciliated. The mouth leads into a very short prepharynx, and this into a powerful muscular pharynx which is 0.3 mm. in cross section. The esophagus follows immediately. The intestinal crura pass back as far as the genital pore. The excretory pore lies on the dorsal surface 0.27 mm. from the posterior tip. The genital pore is on the dorsal surface 0.17 mm. below the excretory pore. The structure of the genital organs is characteristic for the genus Leucochloridium. The cirrus sac is bulbiform or pear shaped, 0.33 mm. by 0.13 mm. It is a connective tissue mass through which passes the ductus ejaculatorius and from which projects the cirrus; the latter is large in cross section and with thick walls. The pars prostatica is short with but few prostatic glands. The tube decreases and, lying free in the parenchyma is found the thick, muscular-walled seminal vesicle. Into this pass the two ducts from the testes which lie on either side of the body, one behind the other. Between them is the small ovary, lying close to the posterior testes. A receptaculum seminis is lacking. Laurer's canal passes posteriorly and dorsalward to end in the excretory vesicle. The yolk glands lie on the side of the body and stretch from the posterior end of the intestinal crura to the level of the pharynx. The uterus follows the general form of the type species. The egg shell is thick, but not very dark colored, measuring 27μ by 15μ.

Lühe (1909) has defined the genus Leucochloridium as follows: Small distomes, thickened at the rounded end, oval in cross section, with well muscled bodies. Large, very strong suckers. Skin ciliated or smooth. Pharynx strong, esophagus very short. Intestinal ceca very thin and reaching to the posterior end. Excretory pore a little distance from the posterior tip on the dorsal surface. Excretory bladder simple, short. Genital opening at the end. Cirrus sac posterior but enclosing the cirrus and ductus ejaculatorius only. The short prostatic part is also muscular, a spindle shaped seminal vesicle lying free in the parenchyma, passes laterally and obliquely to the posterior part of the body from the testes; the ovary lies between the testes. A receptaculum seminis is lacking. Laurer's canal is present. The yolk glands are numerous, and placed at the sides of the body,

lateral to the intestinal ceca. The uterus has numerous coils, entirely encircling the sucker (ventral). Eggs numerous, small with thick shells. Habitat: the cloaca of birds.

LEUCOCHLORIDIUM PROBLEMATICUM

The description of the sporocyst was made from two mature sporocysts which were essentially alike in all respects. The sporocyst of this species is 1.4 cm. long by 0.33 cm. wide and pointed at both ends. The proximal end is continued in a thread-like tube which a little distance away has in its course a small fusiform swelling. The tube then continues into the liver of the snail where many small knob-like projections appear in the hepatic tissue and are connected by thread-like processes. The wall of the sporocyst is rather thick and tough, being 6μ thick. It is white and translucent. The distal half of the sporocyst is banded with deep golden red bands which show very accurately in figure A (Plate). Some of these are darker than others and the lighter ones tend to be more yellow. No bands appear beyond the distal half. The proximal ¼ is flecked with bronze spots, minute in size, but readily seen. These flecks are also present for a short distance on the tube projection. By comparison with the sporocyst of *L. macrostomum,* it will be seen that this sporocyst is essentially different in marking and color. They are both near the same size.

A cross section of the wall of the sporocyst is seen in figure 14. It is made up of an outer longitudinal layer and an inner circular layer of muscles; the former becomes divided by diagonal fibers which serve to break them up into bundles. The nuclei are irregular and branching. Pigment is present in the cells beneath the outer layer. The sporocyst is capable of pulsation, when mature projects from the snail's tentacle, and each contains about 100 larvae which are very active when freed in water; each has a little sac covering it which is quite transparent and has connections with the two suckers. The sac in the living material was 2.6 mm. by 1.4 mm.

The larvae themselves are about 2.2 mm. long and 0.85 mm. wide. They are quite active and very transparent. The cuticula is beset with fine cilia and some of the organ systems are best studied from living material. This form has two well developed suckers which are readily seen with a low power lens. The oral sucker is the larger of the two, and has its opening near the anterior end of the body. This sucker is 0.24 mm. wide and 0.4 mm. deep. Its posterior end leads into a rather powerful muscular pharynx which is 0.17 mm. by 1.15 mm. From this the lateral intestinal crura arise and each one passes down on either side of the worm to the level of the opening of the excretory canal. These crura are not especially narrow as in *L. macrostomum,* but are in *L. problematicum* 0.55 mm. in diameter. The ventral sucker

is 0.34 mm. in cross section, is circular and situated about in the middle of the antero-posterior axis.

The excretory system is not unlike that of *L. macrostomum,* and consists of a rather simple set of tubules. The flame cells are situated throughout the body and seemed to be collected in a larger pair of tubules, one right and one left. These pass down in the body parenchyma on the ventral side of and median to the intestinal crurae to within 0.10 mm. of the end of the crura. Making a rather sharp turn each tubule, expanding in diameter, passes anteriorly and laterally to the crura to within 0.2 mm. of the anterior tip of the body. Another sharp turn here occurs and with increasing diameter each tubule passes posteriorly between the ascending ramus and its corresponding one of the intestinal crura gradually towards the mid line, where it joins its fellow, after a slight fusiform enlargement, in the mid line 0.09 mm. from the posterior tip on the dorsal side of the body. Almost immediately the excretory pore opens to the exterior. These posterior enlargements of the descending rami are pulsating in character, filling and emptying every few seconds.

The genital organs are quite well developed in these larvae and one has no difficulty in outlining them, as they are certain to occur in the adult. The testes are two in number, one anterior lying to the right of the mid line, the other posterior, 0.13 mm. behind the former and to the left of the mid line. There passes from each and towards the other a small duct, the two joining 0.03 mm. from the posterior testis; from this union there arises a small duct which passes posteriorly, slightly to the right of the mid line, through the cirrus sac and opens as the ductus ejaculatorius to the exterior 0.58 mm. from the posterior tip in common with the uterus. The cirrus sac is fusiform, tapering more sharply anteriorly and is 0.16 mm. by 0.07 mm. No cirrus is developed in this stage of the larvae.

The ovary is spherical in shape, 0.052 mm. in diameter and lies on the left side of the body, at a level between the two testes and nearer the posterior one. There passes from it towards the mid line a short oviduct which almost immediately is joined by Laurer's canal. This canal passes posteriorly and ends in the excretory duct immediately before it opens to the exterior, just as described in the case of *L. insignis* by Looss. The oviduct after a short bend receives the two ducts from the embryonic yolk glands. Following this the oviduct makes a twist upon itself and then passes anteriorly as the ascending uterine branch, first in the mid line, then to the left of the ventral sucker. This branch turns toward the mid line anterior to the sucker and passes posteriorly to the right, then in the mid line and by a more or less straight course to the genital pore on the dorsal surface of the body.

The oviduct receives the yolk gland ducts, which are in reality the shell glands, and makes a coil within an organ called the "round body." This is perhaps a gland which acts in some way upon the shell gland substance, perhaps as a precipitin, and was noted in the larva of *L. macrostomum,* but not in the adult.

Several unicellular glands are found just posterior to the ventral sucker and also along the anterior margin of the oral sucker. Their function is one of speculation, and no data is at hand to make even a profitable explanation for their presence.

TABLE OF MEASUREMENTS

	L. macrostomum Larva	L. macrostomum Adult	L. problematicum Larva	L. insignis Adult	L. cercatum Adult
Length	0.8	1.8	2.2	3.0	4.0
Width	0.45	0.9	0.85	1.35	1.2
Anterior sucker	0.17	0.20	0.39	0.73	0.60
Ventral sucker	0.14	0.20	0.34	0.69	0.72
Width pharynx	0.075	0.16	0.15	0.30	0.22
Testes					
Anterior	0.058	0.20	0.074	0.22	0.26
Posterior	0.060	0.22	0.061	0.20	0.26
Ovary	0.059	0.20	0.052	0.13	0.24
Cirrus sac	0.061 by 0.061	0.15 by 0.16	0.07 by 0.16	0.13 by 0.33	0.13 by 0.34
Round body	0.041		0.043		
Larval sac	1.1 by 0.8		2.6 by 1.3		
Sporocyst	1.7 by 0.25 cm.		1.3 by 0.3 cm.		

All measurements in millimeters.

DISCUSSION

In looking over the description given by Looss of *L. insignis* one is struck with the fact that it is very much like the larval form described in this paper, and the question at once arises as to whether this is in reality the larval form of Looss' species. *Fulica atra* is not found in Fairport, but the corresponding American species is, and it is not impossible that these two trematodes are one and the same. The author, however, does not feel warranted in coming to this conclusion chiefly from a geographical reason, although there is nothing definitely present or absent in the larvae to distinguish it from *L. insignis.* I have, therefore, given it a name symbolic of my conception of the species, and it remains yet to find or experimentally develop adults of *L. problematicum,* which may be compared with *L. insignis.*

In addition to this rather perplexing situation one cannot help but note the similarity between the species of Looss and that of Monticelli. Unfortunately, the description of the latter author is not very complete, and if it is correct and the figure is accurate the form may not even be a member of this genus, because, according to him, the ovary is posterior to both testes, while the genus Leucochloridium is characterized by the location of the ovary between the two testes. However, it seems to me that Monticelli has made an incorrect observation on this point, and the organ labeled "posterior testis" is really the ovary. Granting this, the two forms are then very closely related

and come from the same bird genus. The general size of the worms and their organs are very nearly alike, and the shape of the cirrus sacs are also similar. In the absence of detailed information concerning the species and the fact that Looss, being cognizant of the description of Monticelli, considered his different after a study of his own material, makes it hazardous for me to say that these two worms are the same.

It is not possible to say absolutely, therefore, that the larval form *L. problematicum* is or is not the larval form of *L. ceratum* or of *L. insignis,* or still yet whether it is or is not the larval form of both, they being the same species.

CONCLUSIONS AND SUMMARY

1. A larval trematode, from sporocysts found in *Succinea retusa* and *Planorbis trivolvis,* has been described from Fairport, Iowa.

2. This trematode belongs to the genus Leucochloridium, but is unlike the only larval form of this genus ever described. It has been named *Leucochloridium problematicum.*

3. This larva is remarkably like the adult *L. insignis* (Looss) and *L. ceratum* (Monticelli), and it is possible that they are one and the same species.

BIBLIOGRAPHY

Call, R. E. 1898.—A descriptive illustrated catalogue of the Mollusca of Indiana. Report of the State Biologist.

Carus. 1835.—Beobachtung über einen merkwüdigen schöngefärbten, etc. Nova Acta Acad. Caes. Leop. Carol, natur, curios., 17; 85-100.

Heckert, G. A. 1889.—*Leucochloridium paradoxum.* Monographische Darstellung der Entwicklungs und Lebensgeschicte des *Distonium macrostomum.* Biol. Zool., 4:66 pp. 4 pl.

Looss, A. 1899.—Weitere Beiträge zur Kenntniss de Trematoden-Fauna Ægyptens, zugleich Versuch einer natürlichen Gliederung des Genus Distomum Retizus. Zool. Jahrb., Syst., 12:521-784.

Lühe, M. 1909.—Parasitische Plattwürmer. I. Trematodes. Süsswasserfauna Deutschlands, 17:217.

Monticelli, F. S. 1888.—Saggio di una morfolgia dei trematodi. (Habil. Schrift). Napoli, 130.

Stiles, C. W., and Hassall, A. 1898.—An inventory of the genera and subgenera of the trematode family Fasciolidae. Arch. Parasitol., 1:81-99.

Ward, H. B. 1917.—Parasitic flatworms. Ward and Whipple, Fresh-Water Biology, 409-423.

Zeller, E. 1874.—Ueber *Leucochloridium paradoxum* Carus und die weitere Entwickelung seiner Distomenbrut. Zeit. wiss. Zool., 24:564-578; 1 pl.

Abbreviations Used in Plates

a t	anterior testis	*o m*	oblique muscle
a s	anterior sucker	*ov*	ovary
a ut	ascending uterus	*ovd*	oviduct
c	cirrus	*p*	pharynx
c m	circular muscle	*p t*	posterior testis
c s	cirrus sac	*r*	rounded body
d ut	descending uterus	*u g*	unicellular glands
e d	excretory duct	*u t*	uterus
e p	excretory pore	*v d*	vas deferens
g p	genital pore	*v dt*	vitillarian ducts
i	intestinal crura	*v s*	ventral sucker
l c	Laurer's canal	*y g*	yolk glands
l m	longitudinal muscle		

The magnification line by the side of each drawing represents a length of 0.2 mm. in all except Figures 13 and 14, in which it is 0.05 mm. long, and Figures 12 and 23, in which it is 0.1 mm. long.

Explanation of Plate IX

Fig. 1.—Toto drawing of *L. problematicum* from the ventral aspect.

Fig. 2.—Drawing of the reproductive organs of *L. problematicum* from the dorsal aspect. × 150.

Fig. 3.—Toto drawing of the larva of *L. macrostomum* after Zeller. × 120.

Fig. 4.—Toto drawing of the larva of *L. macrostomum* after Carus.

Fig. 5.—Toto drawing of the adult of *L. macrostomum* after Zeller. × 60.

Fig. 6.—A group of young sporocysts of *L. problematicum* dissected from the liver of *Planorbis trivolvis.* × 8.

Fig. 7.—Longitudinal and transverse sections through *L. problematicum.* Longitudinal sagittal section.

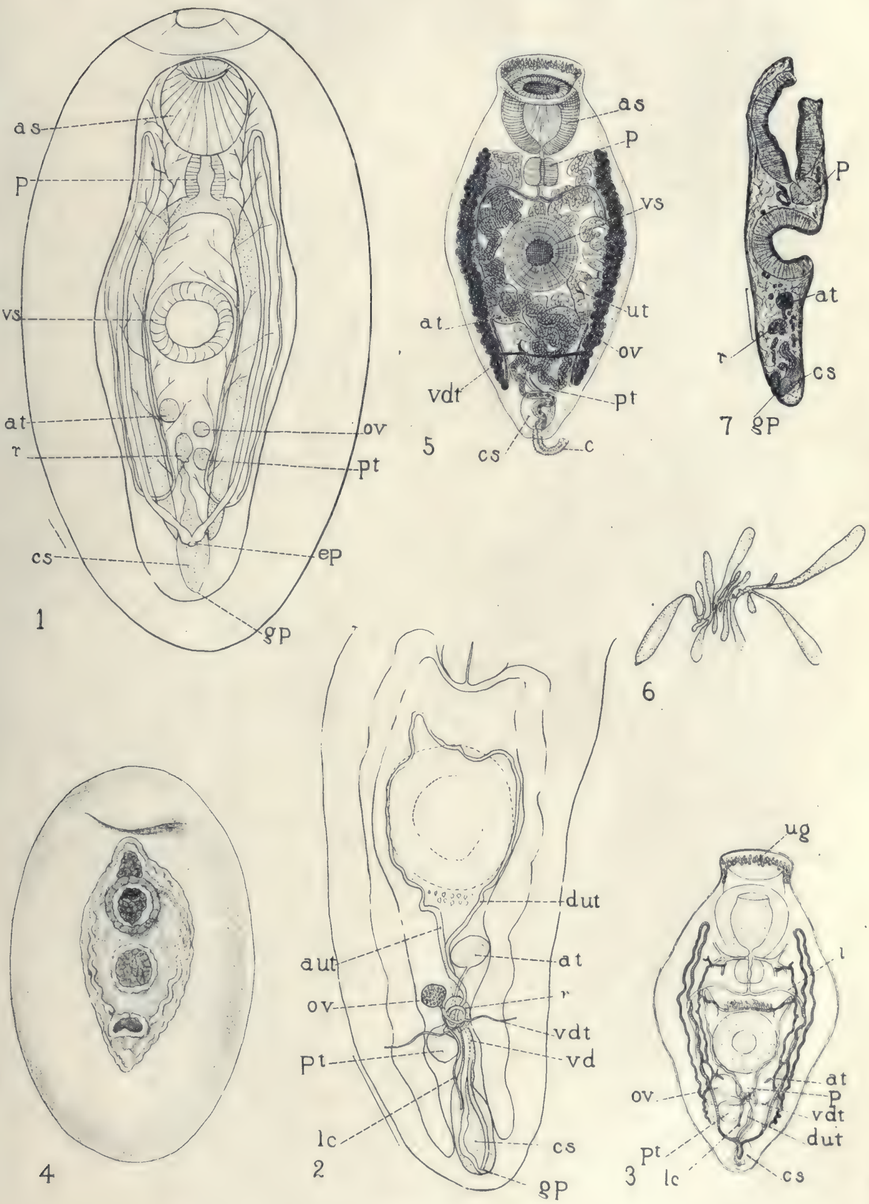
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PLATE IX

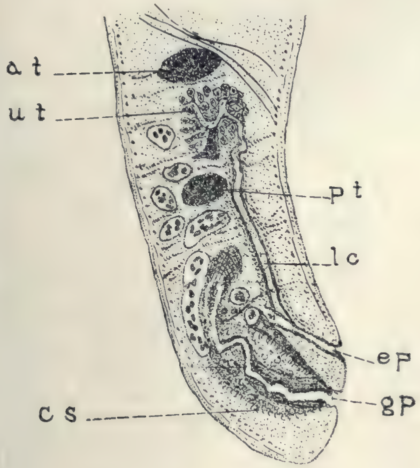
Explanation of Plate X

Fig. 8.—Toto drawing of *L. insignis* after Looss. × 30.

Fig. 9.—Longitudinal sagittal section through the posterior end of the body of *L. insignis,* after Looss.

Fig. 10.—Toto drawing of *L. ceratum* after Monticelli.

Fig. 11.—Posterior region of *L. ceratum,* after Monticelli.



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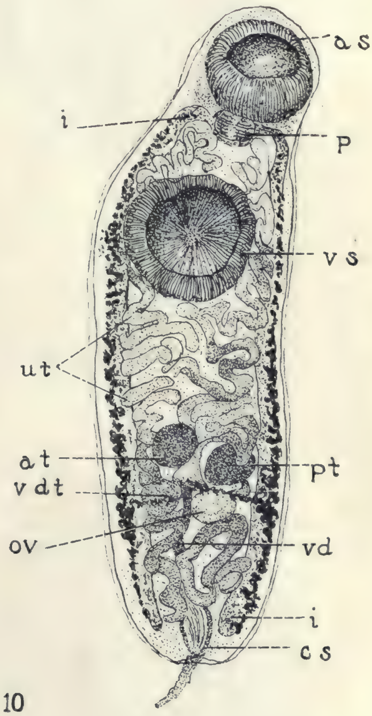

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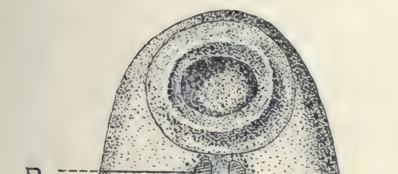

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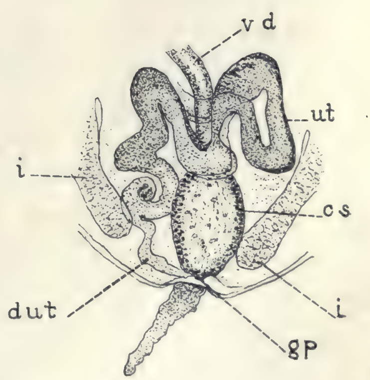

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PLATE X

Explanation of Plate XI

Leucochloridium problematicum

Fig. 12.—Longitudinal sagittal section through the anterior margin.

Fig. 13.—Longitudinal sagittal section through the posterior end of the body.

Fig. 14.—Transverse section through the sporocyst wall.

Fig. 15.—Transverse section through the anterior sucker.

Fig. 16.—Transverse section through the anterior sucker, posterior to Figure 15.

Fig. 17.—Transverse section through the ventral sucker.

Fig. 18.—Transverse section through the pharynx.

Fig. 19.—Transverse section through the anterior testis.

Fig. 20.—Transverse section through the ovary.

Fig. 21.—Transverse section through the rounded body.

Fig. 22.—Transverse section through the posterior testis.

Fig. 23.—Transverse section through the cirrus sac.

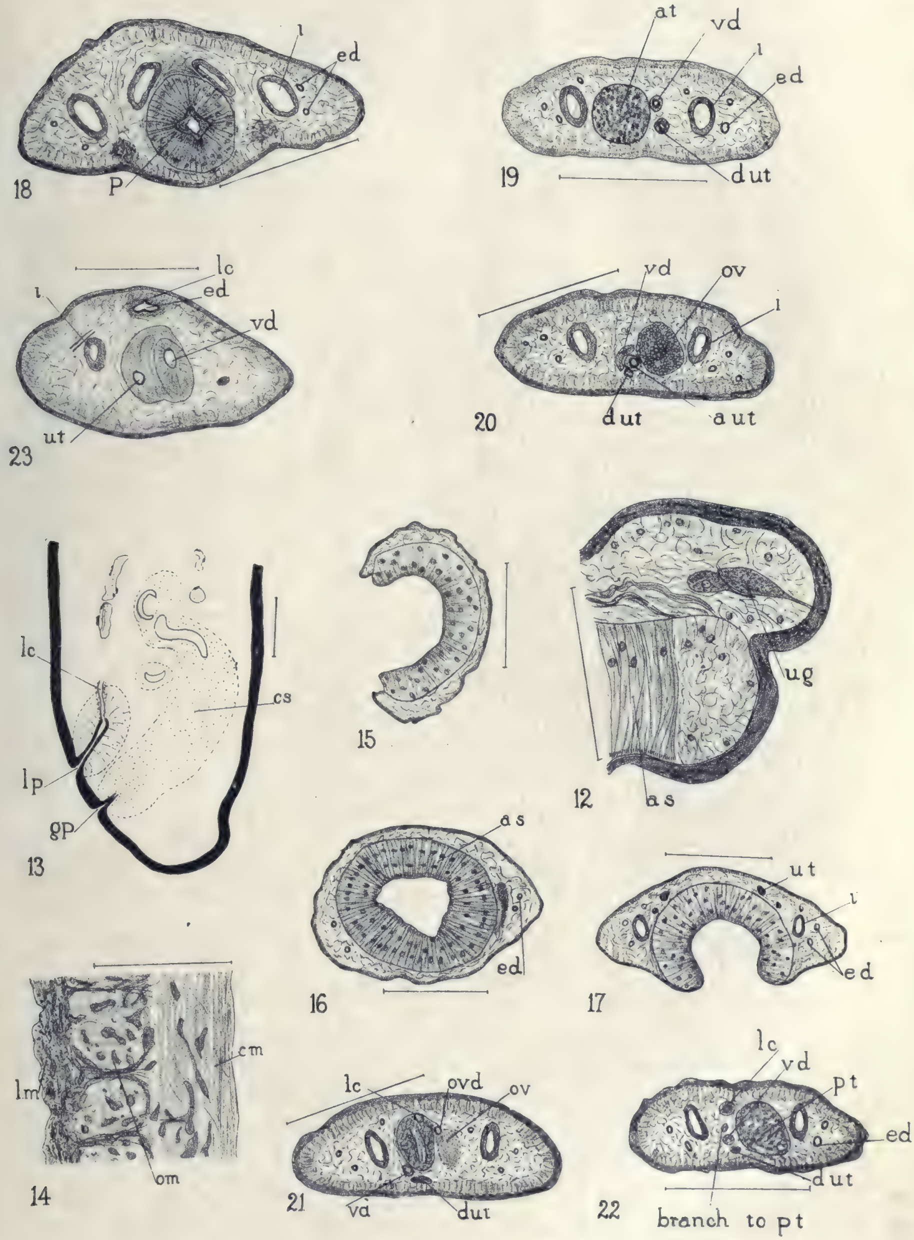
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PLATE XI

THE BIOLOGICAL RELATIONSHIPS OF ASCARIDS

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The experiments described in this paper were undertaken with a view of determining whether, by means of immunological reactions, *Ascaris lumbricoides* which occurs in man can be differentiated from *Ascaris lumbricoides* which occurs in the hog. Morphologically, the forms from the two hosts are indistinguishable so far as present knowledge goes. The name *Ascaris suum* or *Ascaris suilla* which is used by certain writers to designate ascarids which occur in swine is not generally accepted by zoologists for the reason that the classification of animal parasites is based on morphology and not on host relationship. Despite the fact, however, that the specific identity of Ascaris from the hog and from man is commonly accepted on the basis of our present knowledge of the morphology of these forms, much work still remains to be done in order to establish that view beyond any doubt.

The problem which the present writer undertook to solve was whether the apparent morphological identity of *Ascaris lumbricoides* from man and from the hog is correlated with a biochemical identity so far as that can be determined by immunological tests. The solution of this problem necessitated preliminary information as to the possibility of differentiating genera and species of ascarids by immunological methods. The data presented in this paper cover several species of ascarids and throw light on the biological relationships of the forms under consideration.

Flury (1912) made a rather extensive study of the chemistry and toxicology of Ascaris and failed to find any essential differences between *Ascaris lumbricoides* and *Ascaris equorum*, two species that are quite distinct morphologically. Flury employed the methods of analytical chemistry and the usual technic of testing the physiological effects of tissue and organ extracts. The present writer resorted to the more delicate immunological tests by which specific differences may be more readily detected. The differentiation of the fluids of vertebrate species by means of immunological reactions has been studied by many investigators, notably by Nuttall and Uhlenhuth. The former (Nuttall, 1904) writes as follows with reference to the differentiation of the blood of vertebrates by means of cross-precipitin tests:

"The degree and rate of blood reaction appear to offer an index to the degree of blood relationship; in other words, closely related bloods react more powerfully (more precipitum) and more rapidly than do distantly related bloods, provided the latter react at all."

EXPERIMENTS WITH PRECIPITIN TESTS

As is well known the blood serum of an animal immunized to solutions containing proteins acquires the power of precipitating these proteins from solution. Rabbits are commonly used for the purpose of obtaining precipitating serum, and injections are made at intervals of about six days. Four or five injections are usually sufficient to produce a rich precipitin content in the serum.

Following the above technic the present writer immunized a number of rabbits to physiological salt-solution extracts of *Ascaris lumbricoides* from swine. The extracts were made by adding to salt solution pulverized material of entire worms, dried at room temperature shortly after they were removed from the host and washed in physiological salt solution, and extracting for a day or more at room temperature. After filtering the extracts they were preserved with a sufficient quantity of carbolic acid to make a 0.25 per cent. solution. Rabbits were injected intravenously and were bled about a week after the last injection. Small quantities of blood were obtained by severing the marginal ear vein under aseptic precautions. Larger quantities of blood were drawn directly from the heart under ether anesthesia.

Owing to the scarcity of material other than *Ascaris lumbricoides* from swine precipitating serum prepared by immunizing rabbits with extracts of that species only was used. The serum was tested against extracts of several species of ascarids as noted below. The extracts for the tests proper were prepared by adding a definite quantity of dried-worm material to a definite quantity of physiological salt solution and allowing it to extract for a day or longer in a refrigerator. When ready for use the extracts were filtered several times through ordinary filter paper until the filtrates were clear. In each series of tests similar quantities of coarsely pulverized material from each species were extracted in equal quantities of physiological salt solution at the same time and under identical conditions.

Following are the results of the first series of experiments:

The extracts employed in these tests were prepared on the basis of 100 mgm. of dry worm material to 2.5 c.c. of physiological salt solution. The precipitating serum which was used was rather weak.

Five drops of serum were added to tubes containing, respectively, five drops of extract of the following species: *Ascaris lumbricoides* (from swine), *Ascaris equorum, Belascaris marginata,* Toxascaris species (from a wild cat), *Ascaridia maculosa.*

The tube containing an extract of *Ascaris lumbricoides* showed a heavy precipitate in about 20 minutes. The contents of the tube containing an extract of *Ascaris equorum* showed marked clouding two hours after the serum had been added. This was followed by the

settling of a precipitate. The bulk of the precipitate was considerably less than that which settled in the tube containing an extract of *Ascaris lumbricoides.* The contents of the tubes containing extracts of Belascaris and Toxascaris were found to show cloudiness at about the same time, approximately two hours after the serum had been added. The amount of the precipitates formed in these tubes was smaller than that formed in the tube containing the extract of *Ascaris equorum.* The tube containing the extract of *Ascaridia maculosa* remained clear for about four hours during which it was kept under observation. An examination of the contents of the tube the following day showed a very light precipitate, much smaller in amount than those present in the tubes containing extracts of Belascaris and Toxascaris.

These experiments were repeated by using larger quantities of fluids, namely, 25 drops of extracts and 10 drops of serum. After adding the serum the tubes were placed in an incubator at a temperature of 37° C. After 15 minutes' incubation the tube containing the extract of *Ascaris lumbricoides* showed a marked precipitate. The tubes containing extracts of the other species were clear. After being taken out of the incubator the tubes were kept under observation over an hour, but no precipitates developed. The following day precipitates were found in all tubes. Judged by the quantity of precipitate present the tubes ranged in the following descending order: *Ascaris lumbricoides, Ascaris equorum,* Toxascaris, Belascaris and Ascaridia. The tube containing an extract of Ascaridia showed but a slight precipitate.

Additional experiments were carried out with serum diluted in physiological salt solution. Five drops of a 50 per cent. dilution of serum added to five drops of extract of each species referred to above yielded practically the same results as those obtained with pure serum except that precipitates were not noted in the tubes containing extracts of *Ascaris equorum,* Belascaris, Toxascaris and Ascaridia during the period that they were kept under observation (about four hours), while the contents of the tube containing an extract of *Ascaris lumbricoides* became cloudy in about 30 minutes and showed a marked precipitate 30 minutes later. An examination of the tubes the following day showed the presence of precipitates in all tubes except in that containing an extract of Ascaridia. The bulk of precipitate was greatest in the tube containing an extract of *Ascaris lumbricoides.* The tube containing an extract of *Ascaris equorum* was next in order, while that containing an extract of Belascaris showed the smallest quantity of precipitate. Five drops of a 30 per cent. dilution of serum added to five drops of extract of *Ascaris lumbricoides* caused the appearance of cloudiness followed by the formation of a precipitate in less than an hour. Extracts of other ascarids were not tested with this dilution of serum.

Further tests were made primarily with a view of obtaining more data on the differences in the degree and rate of reaction between extracts of *Ascaris lumbricoides* and *Ascaris equorum* by using similar extracts of the two species with equal quantities of serum. The results were uniformly the same, namely, a heavier and more rapidly appearing precipitate in tubes containing extracts of *Ascaris lumbricoides* than in those containing extracts of *Ascaris equorum.*

Each experiment and series of experiments was controlled as follows:

1. Extract of parasite plus a few drops of salt solution.
2. Precipitating serum plus a few drops of salt solution.
3. Normal serum plus a few drops of extract tested.

Unless the controls remained clear the results of the test or of the series of tests were disregarded. As a control on the general specificity of the test for ascarids, precipitating serum was tested against an extract of an unrelated form, namely, *Dictyocaulus filaria,* with negative results.

Inasmuch as the experiments which have just been summarized showed quite conclusively that extracts of the two species of the same genus, namely, *Ascaris equorum* and *Ascaris lumbricoides,* can be easily differentiated by the precipitin test the writer carried out a series of experiments at a later date to determine whether extracts of *Ascaris lumbricoides* from man can be differentiated by the same test from similar extracts of *Ascaris lumbricoides* from the hog. As a control on extracts of these forms an extract of *Ascaris equorum* was tested at the same time. The three extracts were prepared in a similar way, namely, by adding 0.3 gm. of coarsely powdered worm material from each host to 5 c.c. of physiological salt solution and allowing the mixtures to remain in a refrigerator for three days. The precipitating serum used in these tests was stronger than that used in the preceding experiments. The extracts were therefore diluted before being tested, since in the concentrated state differences between the rate of reaction of extracts of *Ascaris lumbricoides* and *Ascaris equorum* were lost.

The extracts were diluted from three to five times with physiological salt solution and 10 drops of extract were tested against 1 and 2 drops of serum, respectively. The tube containing an extract of *Ascaris equorum* did not show any precipitate until at least an hour after the addition of the serum, whereas the tubes containing extracts of *Ascaris lumbricoides* from the two hosts showed precipitates in a few minutes. No differences could be detected in the rate of the appearance of these precipitates, but as a rule the precipitates in the tubes containing extracts of *Ascaris lumbricoides* from swine were somewhat heavier than those in the tubes containing extracts of *Ascaris*

lumbricoides from man. It is doubtful, however, whether that fact has any significance in view of the rather crude manner in which the writer was obliged to prepare his extracts. Since material of *Ascaris lumbricoides* from man was scarce it was necessary to weigh out small quantities which were extracted in correspondingly small quantities of salt solution. It is possible that certain parts of the worm are more soluble in salt solution than others, so that when material from one or two specimens is used the quantity of extract obtained is less than when a similar quantity by weight is extracted from fragments of many specimens. Probably a more accurate method of performing the test would be to use the coelomic fluid of the worms instead of salt-solution extracts. It is expected that as soon as fresh material of *Ascaris lumbricoides* from man is available additional experiments will be undertaken to secure further data on that point.

These experiments were repeated by using more dilute extracts. Thus a dilution of each extract made by adding one part of the extract to nine parts of physiological salt solution and testing 10 drops of the diluted extract against 1 and 2 drops of serum, respectively, yielded the following results: After one hour the contents of the tubes containing extracts of *Ascaris lumbricoides* from the two hosts became cloudy, whereas the tube containing an extract of *Ascaris equorum* was perfectly clear. An examination of these tubes on the following day showed marked precipitates in those containing extracts of *Ascaris lumbricoides* from the two hosts and a slight precipitate in the tube containing an extract of *Ascaris equorum.* A still greater dilution of the extract, namely, 19 parts of salt solution to one part of extract yielded similar results; that is, the tubes containing extracts of *Ascaris lumbricoides* from the two hosts showed precipitates in about three hours, whereas the tube containing an extract of *Ascaris equorum* did not show a precipitate in eighteen hours.

The precipitating serum used in the above-mentioned series of experiments was tested against extracts of Toxascaris species and *Strongylus vulgaris* by adding equal quantities of serum to each extract. Inasmuch as the worm material which was extracted for these experiments was small in bulk the extracts were rather dilute. No precipitate appeared in the tube containing an extract of Strongylus after twenty hours. A very slight precipitate was seen in the tube containing an extract of Toxascaris after a similar period. An extract of *Ascaris lumbricoides* of approximately the same strength as those of the two parasites referred to above, plus an equal quantity of serum, showed a well-marked precipitate about an hour after the serum had been added.

All experiments in this series were controlled as has already been noted elsewhere in this paper.

Summarizing the results of the experiments concerning the relationship of the species of ascarids considered in the foregoing pages, it may be stated that the results of the precipitin tests correspond to the known zoological relationships of these parasites. The differences in the degree and rate of reaction between extracts of two species of the same genus are less than those between extracts of different genera. The slight reactions obtained with extracts of Ascaridia are decidedly significant in view of the fact that that genus is more distantly related to the genus Ascaris than are the genera Belascaris and Toxascaris. The two latter genera are included with Ascaris in the family Ascaridae, whereas the genus Ascaridia belongs to the family Heterakidae. These two families are included in the same superfamily, namely, Ascaroidea. No less significant is the failure to obtain any precipitates with extracts of Dictyocaulus and Strongylus, genera belonging to the superfamily Strongyloidea.

EXPERIMENTS WITH THE ANAPHYLACTIC TEST

The experiments with the precipitin test were supplemented by another series of immunologic tests, namely, by the anaphylactic reaction. The latter is based on the fact that an animal that has received an injection of protein material develops after a certain period a condition of hypersusceptibility to the protein or proteins in question, or, in other words, becomes sensitized to the protein or proteins. A reinjection of material similar to that used in the sensitizing injection calls forth a series of more or less grave symptoms which frequently terminate in death. The anaphylactic reaction is independent of the toxicity of the material injected, since it may be produced by substances that are nontoxic to normal animals.

Without describing in detail the exact response observed by the writer in guinea-pigs sensitized to very small quantities of extracts of various ascarids and reinjected after a period of incubation of two weeks or longer with extracts of the same species as that used for the sensitizing injection and with extracts of related species, the results of several series of experiments involving eighteen guinea-pigs will be summarized briefly. It is important to state in this connection that as a result of the work conducted by various members of this laboratory during the past two years, it was found that guinea-pigs are very tolerant of rather heavy single injections of the body fluids of *Ascaris lumbricoides*. These observations are in harmony with a considerable amount of published data on the effects of the body fluids of ascarids on various animals. The reactions observed by the present writer were considered to be, therefore, anaphylactic reactions and not reactions to any toxic constituents which the fluids of these parasites may contain.

For purpose of convenience the reactions will be referred to as follows:

Mild.—General body tremor, rapid breathing, muscular twitching, scratching of face, etc.

Marked.—In addition to the symptoms above, weakness in legs, frequent defecation and urination, tendency to fall down, etc.

Severe.—In addition to above symptoms, general paralysis.

The results of the experiments follow:

Series A. Six guinea-pigs were sensitized to a salt-solution extract of *Ascaris lumbricoides* from swine by a subcutaneous injection of 0.2 c.c. of an extract made by adding 0.5 gm. of powdered worm material to 15 c.c. of physiological salt solution and allowing the powder to extract for about two hours at room temperature. Fourteen to fifteen days later the animals were reinjected intraperitoneally with 2 c.c. of more concentrated extracts than that used for the sensitizing injection. The results follow:

No. 1. Reinjected with an extract of *Ascaris lumbricoides* from swine. Reaction mild.

No. 2. Reinjected as No. 1. Reaction mild.

No. 3. Reinjected with an extract of *Ascaris lumbricoides* from man. Reaction mild.

No. 4. Reinjected with an extract of Belascaris. No reaction.

No. 5. Reinjected with an extract of *Ascaridia maculosa.* No reaction.

No. 6. Reinjected with an extract of *Ascaris equorum.* No reaction.

Series B. The guinea-pigs used in this series were sensitized by subcutaneous injection of 0.5 c.c. of salt-solution extract of *Ascaris equorum* of about the same concentration as that used in sensitizing the animals of series A. Twelve days after the sensitizing injection the guinea-pigs were reinjected with 2 c.c. of more concentrated extracts as follows:

No. 7. Reinjected with extract of *Ascaris equorum.* Severe reaction.

No. 8. Reinjected with an extract of *Ascaris lumbricoides* (from swine). Marked reaction.

No. 9. Reinjected with an extract of Belascaris. Slight reaction.

Additional experiments were performed as follows:

No. 10. Sensitized to an extract of *Ascaris lumbricoides* (from man). Reinjected twelve days later with an extract of *Ascaris equorum.* Mild reaction.

No. 11. Sensitized to an extract of Belascaris and reinjected twelve days later with an extract of *Ascaris lumbricoides* (from swine). Reaction mild.

No. 12. Sensitized as No. 11 and reinjected thirteen days later with an extract of *Ascaris lumbricoides* (from man). Reaction mild.

No. 13. Sensitized as No. 11. Reinjected with an extract of Toxascaris thirteen days later. No reaction.

No. 14. Sensitized to an extract of *Ascaris lumbricoides* (from man). Reinjected with an extract of *Ascaris lumbricoides* (from swine) twelve days later. Mild reaction.

Series C. The guinea-pigs used in this series were sensitized to an extract of *Ascaris lumbricoides* (from swine). The extract employed for the sensitizing injections was prepared in a similar manner as that described for Series A. From 0.2 to 0.5 c.c. were used in the sensitizing injection which was given subcutaneously. The animals were reinjected eighteen days later with 1 c.c. of concentrated extracts as follows:

No. 15. Reinjected with an extract of *Ascaris equorum*. Severe reaction.

No. 16. Reinjected with an extract of *Ascaris equorum*. Severe reaction.

No. 17. Reinjected with an extract of *Ascaris lumbricoides* from swine. Fatal, death occurring forty minutes after the injection.

No. 18. Reinjected as No. 17. Mild reaction.

The results of experiments with the anaphylactic reaction are not so constant as the results of the precipitin tests, due, no doubt, to the fact that the latter take place in test tubes, whereas the former take place in living animals. Furthermore, the number of animals used is scarcely sufficient to justify any definite conclusions. In a general way, however, more marked reactions were obtained when a guinea-pig was reinjected with an extract of the same species as that used for the sensitizing injection than when it was reinjected with an extract of a related species. It is interesting to observe, also, that the guinea-pigs in series A, which were evidently only slightly sensitized to ascarid extracts, reacted to extracts of *Ascaris lumbricoides* from the two hosts in practically the same way, but that those that were reinjected with extracts of other species gave no reaction.

Attempts were also made to test the series of extracts of ascarids by means of the complement-fixation reaction, but in view of the fact that the extracts employed exhibited a marked tendency to yield non-specific complement fixation and that rabbit serum frequently exhibits anticomplementary properties, that phase of the work was temporarily abandoned.

SUMMARY AND CONCLUSIONS

1. The blood serum of rabbits immunized to salt-solution extract of *Ascaris lumbricoides* (from swine) causes the formation of precipitates when added to salt solution extracts of various ascarids (Ascaris, Belascaris, Toxascaris, Ascaridia). The precipitin reaction as applied to extracts of these parasites is therefore a group reaction.

2. By the use of proper dilutions heavier and more rapidly appearing precipitates are produced when rabbit serum immunized against *Ascaris lumbricoides* is added to salt solution extracts of *Ascaris lumbricoides* than when it is added to similar extracts of other ascarids. Extracts of species of the same genus (*Ascaris lumbricoides* and *Ascaris equorum*) show less difference in that respect than extracts of worms belonging to different genera (Ascaris, Belascaris, Toxascaris, Ascaridia). The results of the precipitin tests correspond, therefore, to the zoological relationships of these parasites.

3. Extracts of *Ascaris lumbricoides* from man do not appear to be distinguishable from extracts of *Ascaris lumbricoides* from swine so far as the results of the precipitin test are concerned. Apparently, the forms from the two hosts are biochemically as well as morphologically indistinguishable.

4. Small quantities of precipitating serum sufficient to cause the formation of precipitates in salt-solution extracts of ascarids failed to produce precipitates in similar extracts of unrelated nematodes (Dictyocaulus, Strongylus).

5. The results obtained by means of the anaphylactic test appear to be in a general way in agreement with the results of the precipitin test, although a considerable degree of variation was noted as regards the reactions of guinea-pigs to injections of similar extracts. Definite conclusions from the experiments on anaphylaxis are not justified in view of the limited number of experiments.

Literature Cited

Flury, Ferdinand. 1912.—Zur Chemie und Toxikologie der Ascariden. Arch. exper. Path. u. Pharmakol., 67: 275-392.

Nuttall, George H. F. 1904.—Blood immunity and blood relationship. Cambridge [Eng.].

THE FLAGELLATE CHARACTER AND RECLASSIFICATION OF THE PARASITE PRODUCING "BLACKHEAD" IN TURKEYS—*HISTOMONAS* (GEN. NOV.) *MELEAGRIDIS* (SMITH)*

Ernest Edward Tyzzer

The demonstration of the constant presence in the parasite of Blackhead of an extranuclear body which takes part in nuclear division, and the occurrence of a type of nuclear division commonly found in trichomonads (Kofoid and Swezy, 1915) led to the suggestion in an earlier paper (Tyzzer, 1919) of its flagellate character. A subsequent series of observations, to be recorded further on in the present paper, show that this organism may exhibit under certain conditions characteristic flagellate motility.

In the paper above referred to, the author has called attention to the extreme pleomorphism of the protozoon in question, the extent of its morphological variation having no parallel among known parasitic amoebae. The distribution of the various forms of the parasite bears such a relation to the age of the pathological process with which they are associated that they may be interpreted as representing phases of development. Amoebiform organisms with clear blue staining cytoplasm, either with or without inclusions of the nature of ingested material, which occur in great numbers in early lesions and at the periphery of older lesions, were considered to be invasive forms. Organisms having a clear, faintly staining cytoplasm, frequently occurring in such numbers as to greatly distend the tissues and associated with the stage of the disease immediately following invasion were regarded as vegetative forms, although it is quite possible that a large proportion of such organisms present abnormal development and degeneration. A third type of organism found predominating in the older portions of the lesions was interpreted as representing a resistant phase of development. These are relatively small and rounded, present a dense cytoplasm having a marked affinity for eosin and possess a delicate limiting membrane. They are taken up in great number by giant cells.

The multiplication of this organism in the tissues appears to be accounted for by binary fission, at least no evidence of any other type of propagation has been obtained. The process of nuclear division is of the type found in trichomonads. The division centers are derived

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from an extranuclear body, and are connected with each other by a well developed rod-like paradesmose. No multinucleated forms have been found. Parasites with disk-like bodies in the cytoplasm have been interpreted by Smith (1915) as probably representing multiple agamic division. He evidently concludes that this species multiplies by a process which bears a striking similarity to the endogenous spore formation described in amoebae and Balantidium by Walker (1908). Organisms containing these disk-like inclusions are of common occurrence and have been carefully studied in the course of the present investigation. These inclusions vary in size from barely visible particles to bodies exceeding the nucleus in size, and are in some instances so uniform that the parent organisms bear considerable resemblance to the multinucleated cysts of entamoebae. Since the organisms in which they occur retain their original nucleus and extranuclear body unchanged, and since no evidence has been obtained that these disk-like bodies develop into mature organisms, it has appeared more probable that these inclusions represent either the shadows of ingested material or globules of coagulable material.

The dissemination of the parasite from the primary lesions in the ceca to the liver of the turkey is accounted for by Smith through the transportation within phagocytic cells of the problematical small forms discussed above. It is supposed that these cells, after taking up the small forms of the parasite, may in certain instances pass into the veins when they are carried through the portal system to lodge in the liver. It is difficult on this hypothesis to account for the retention of the organisms in the liver, for it would be reasonable to expect that such small parasites on escaping from the phagocytic cells would be occasionally at least swept in the circulation to the lungs and other organs. That the liver serves as an effective filter for the parasite is shown by the limitation of the secondary lesions of the disease to this organ. The demonstration of an active invasive form of the parasite capable of migrating through any of the softer tissues makes the foregoing hypothesis unnecessary. The parasite infiltrates the involved tissue so extensively that it is inconceivable that the blood vessels should escape, and in fact, organisms have not infrequently been found beneath the endothelium of the veins. The size and physical peculiarities of these invasive forms evidently prevent their passage through the capillaries of the liver.

An attempt has been made by Hadley to show that the parasite of Blackhead is identical with "Trichomonas," an intestinal flagellate, which is treated as a species without further distinction. Apparently artefacts and postmortem changes are interpreted as evidences of invasion of the intestinal mucosa by intestinal flagellates. Not only are intestinal trichomonads incorporated into the life cycle of the

parasite, but also organisms of the Blastocystis type. This author has not succeeded in demonstrating intermediate stages connecting any of the intestinal flagellates with the tissue parasite. The confusion of several intermingled species as the developmental forms of a single species has, strange to say, led him also to a conclusion which is not greatly at variance with what is now appparent concerning the nature of the parasite of Blackhead.

Amoeboid movement with slow change of shape has already been recorded (Tyzzer, 1919), but these earlier observations were made upon material kept in a warm chamber at 38 C., rather than at the body temperature of the turkey. Smith (1915) states that no motility has been observed in parasites studied from time to time in the warm chamber except on one occasion when slight changes of form were observed. During the present season, a series of observations made upon fresh material kept at temperatures ranging from 41 to 42 C., the body temperature of the turkey, has brought to light great activity in certain forms of the parasite. The organisms, in hanging drop preparations of scrapings from the lesions mixed with Locke's solution, remain motile for many hours at this temperature. Different types of organisms are distinguishable in fresh material. The small sized dense forms such as are encountered within giant cells are most conspicuous, but these are in general nonmotile. Large and moderate sized forms with a more or less granular cytoplasm frequently show motility, but others appear degenerated and consist of little more than a vesicle containing a granular mass in which a nucleus is at times distinguishable. Least conspicuous are the clear hyaline forms, and these show the greatest activity. The nucleus is usually distinguishable and an occasional organism is found in which four or five lines radiating from the extranuclear body are plainly visible. There is no sharp distinction between ectoplasm and endoplasm, but when granules are present, they are confined to the vicinity of the nucleus, leaving the cytoplasm otherwise clear.

The motility varies in type not only in different organisms, but in the same one at different times during the period of observation, and may be either amoeboid or pulsating in character. Furthermore, the amoeboid movements may be either continuous or interrupted. In the former case the motility may amount in some instances to a slow change of shape, in others to a more or less continuous flowing of the cytoplasm with snail-like progression. In case the amoeboid movement is discontinuous in type, pseudopodia are extended from the main mass of protoplasm, and the latter may stream into the former. There is extreme variation in the rate of motility. The pseudopodia may be sheet-like with a smooth border, but often show one or several rather sharp processes or spurs and are commonly quite irregular. Their

protrusion is usually quite rapid, but not typically eruptive in character. The pseudopodia may be quickly protruded and retracted. An organism watched over a long period of time has been observed to spread out and become sheet-like. Several pseudopodia may be extended almost simultaneously from various points of the surface. Some large organisms may develop a number of short wave-like projections, but subsequently become rounded and remain quiescent. Some maintain a remarkable degree of activity for long periods of time, and amoeboid movement has been noted in organisms from a lesion kept at room temperature for forty-eight hours. Activity in a degree not observed in parasitic amoebae is frequently observed. Sudden displacement or rotation of the main body of organisms frequently results from the movements of extended pseudopodia or adjacent tissue cells may be forcibly separated and shoved to one side. Occasionally an organism exhibits for a considerable period, cytoplasmic movement of a wave-like character, in appearance suggestive of the boiling of a viscid material.

Rhythmic pulsating movements similar to that of trichomonads have been repeatedly observed. This was first noted in a lesion which had been left over night in a partially dissected turkey. Since the possibility of contamination with intestinal flagellates could not be excluded, this observation was disregarded. Organisms showing similar rhythmic movements were subsequently noted in secondary lesions removed with aseptic precautions from several different birds. Stained sections of these lesions reveal no organisms having the morphology of the common intestinal flagellates. Pulsation was in no case immediately apparent in material obtained from freshly killed birds, but developed after a period of from two to four hours in the warm chamber. Several organisms showing this type of motility were in one instance found with the low power from the movement imparted to surrounding cells and debris. They had the morphology of other typical Blackhead parasites present in the material, but were rotating in a jerky manner. No well developed undulatory membrane or flagella were apparent and the rhythmic movement appeared to be internal. On another occasion an organism was observed from which were protruded rather sharp wave-like pseudopodia. These after a time travelled in one direction over the surface of the body, the movements then became characteristic of those of an undulating membrane and the organism began to rotate rather rapidly. Much of the rhythmic movement observed appeared to consist of movement of the interior of the organism, rather than of the surface or of special appendages. Internal granules and nucleus in such instances oscillate with the pulsations without notable change of position of the organism as a whole and without movement of surrounding material. In one case an organ-

ism showing strong, fairly regular rhythmic movement within, continued to send out pseudopodia in various directions.

All attempts to cultivate the organism have thus far failed. The changes taking place after inoculation into the most favorable medium at present available, have been carefully followed. For thirty hours motility continues unimpaired, and the great number of motile forms gives the impression that multiplication is taking place. Binucleate forms are noted and an occasional pair of organisms suggesting binary fission, but there is subsequently no definite increase in number. Actively motile forms occur, but in progressively smaller number up to fifty-four hours in the culture tube at 37 C., but after seventy-two hours all movement has ceased. During this time the amoeboid forms, a large proportion of which show cytoplasmic granules and occasionally rather refractive globules or vacuoles, are replaced by small sized, nonmotile, spherical forms, which possess a clear hyaline cytoplasm without granules or other inclusions and a fairly readily distinguishable nucleus. It is difficult to determine whether the motile forms give rise to the hyaline resting forms or whether they die off, leaving only the latter present. The former possibility appears plausible at least as there are all transitions between the two types. The small nonmotile hyaline forms show at this time no evidence of degeneration or disintegration. No form of the parasite is to be found in culture tubes kept for five days at 37 C., although fairly well preserved organisms are present at this time in tubes kept at room temperature. These, however, show no motility when placed in the warm chamber at 41 or 42 C., and are evidently dead. Notwithstanding the great number of organisms showing amoeboid movement in the case followed in culture medium, the flagellate type of motility was not observed under the conditions furnished. With the appearance of bacteria in the culture, both motile and resting forms of the parasite quickly disappear even though previously present in great numbers. There is no trace of the parasite after twenty-four hours' incubation in salt solution to which a loopful of the cecal contents is added. The prompt disappearance of the organism in liver lesions undergoing putrefaction is also notable.

These observations are in agreement with previous morphological findings and show quite conclusively that the parasite of Blackhead is a flagellate. The rhythmic agitation of internal structures observed in certain instances may be explained only by the presence of a kinetic apparatus wholly enclosed in the cytoplasm. This in all probability consists of the previously described extranuclear body and radiating filaments which may now be interpreted as centroblepharoplast and intraprotoplasmic flagella. It is a notable fact that one of these filaments is coarser than the others (see Figs. 3 to 7, Tyzzer, 1919), but

whether this represents parabasal body or trailing flagellum remains to be determined. Nothing is known concerning the possible existence of free living flagellated forms of this species, although their occurrence is suggested by the development of pulsating movements in organisms observed in the warm chamber. The recognition of such forms might possibly help to solve the problem of its transmission from host to host. It may at present only be conjectured whether such forms exist or whether the organism has become so adapted to parasitic life that it has for the most part lost its ability to live in flagellate form. Wherever, in histological preparations, it has been encountered on mucous surfaces, there is no apparent acquisition of the more characteristic flagellate type of structure. Although pulsating movement characteristic of trichomonads has been observed in material obtained from noncontaminated secondary lesions of several cases, it was not observed in similar material from another case in which amoeboid movement was maintained for fifty-four hours. The exacting conditions necessary to support this organism for even a brief period outside the body of its host as well as its prompt destruction by bacteria indicate that it possesses no marked resistance to conditions encountered outside the body of its host.

Classification.—Smith originally placed the parasite of Blackhead, on account of its amoeba-like characteristics, tentatively in the genus *Amoeba,* and much later (Smith, 1915) retained the same generic name under a different spelling *Ameba.* The view expressed by Hadley that this organism is identical with a previously described coccidium, *Eimeria avium,* is untenable, and was later abandoned by this author. Doflein's suggestion that the organism as a parasitic amoeba should be included in the genus *Entamoeba* now fails to apply with the discovery of flagellate characteristics. Both Jowett's (1911) and Hadley's (1916, 1917) incorporation of the parasite into the genus *Trichomonas* appears to be based upon a confusion of at least two intermingled species for a single species and is unacceptable without more conclusive evidence.

The proof that this organism is not an amoeba makes necessary its reclassification. Its trichomonad affinities are indicated by the type of nuclear division which it presents, by the number of flagella indicated in the five lines radiating from the blepharoplast and by the character of its pulsating movements which appear under certain conditions so that it may thus be included in the family *Tetramitidae* Saville Kent, 1880, as modified by Chalmers and Pekkola, 1918. The assumption of amoeba-like characters with respect to both movement and ingestion of solid particles together with its ability to invade vertebrate tissues appear to justify the creation of a new genus for this species. In case it should prove to be an aberrant form of a type

species already described, the generic name here offered may then be suppressed. The name *Histomonas* is proposed for this genus, which may be defined as follows:

HISTOMONAS gen. nov. Pleomorphic parasitic Tetramitidae with amoeba-like phases of development within tissues of host. The kinetic structures, associated with blepharoplast, intraprotoplasmic during amoeba-like phase. Nuclear division trichomonad in type with well developed paradesmose.

Apart from the pulsating forms in hanging drop preparations of material from lesions, flagellated stages are unknown. No contractile vacuole, no cytostome observed.

Type species: *Histomonas meleagridis* (Smith, 1895) Tyzzer, 1919.

Syn. — *Amoeba meleagridis* Smith 1895.
Eimeria avium Hadley 1909.
Entamoeba meleagridis Doflein 1911.
Trichomonas eberthi Jowett 1911.
Ameba meleagridis Smith 1915.
Trichomonas Hadley 1916.

REFERENCES

Chalmers, A. J., and Pekkola, W. 1919.—*Dicercomonas soudanensis.* Jour. Trop. Med., 22:29.

Hadley, P. B. 1916.—The rôle of the flagellated protozoa in infective processes of the intestines and liver. Bull. 166, Agr. Exp. Sta. Rhode Island.
1917.—The case of trichomonas. Amer. Nat., 51:209.

Jowett, W. 1911.—Blackhead. Infectious entero-hepatitis or typhlo-hepatitis, a disease of young turkeys. Jour. Comp. Path. and Therap., 24:289.

Kent, S.—A manual of infusoria. 2 vol. London, 1880-82.

Kofoid, C. A., and Swezy, O. 1915.—Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad., 51, No. 6.

Smith, T. 1915.—Further investigations into the etiology of the protozoan disease of turkeys known as blackhead, entero-hepatitis, typhilitis, etc. Jour. Med. Research, 33:243-270.

Tyzzer, E. E. 1919.—Developmental phases of the protozoon of "Blackhead" in turkeys. Jour. Med. Research, 40:1-30.

Walker, E. L. 1908.—The parasitic amoebae of the intestinal tract of man and other animals. Jour. Med. Research, 17:379-459.

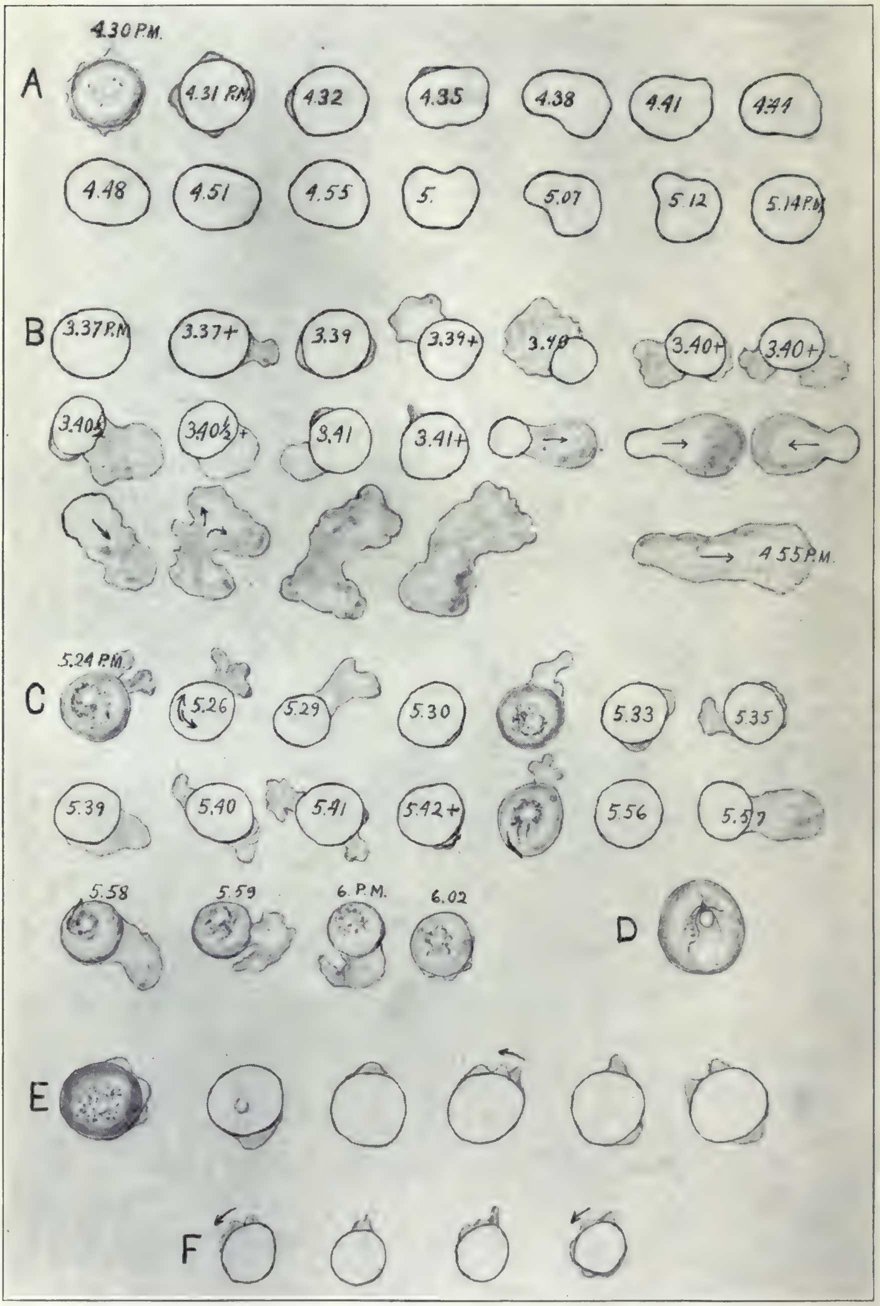
A
4.30 P.M.
4.31 P.M.
4.32
4.35
4.38
4.41
4.44
4.48
4.51
4.55
5.
5.07
5.12
5.14 P.M.
B
3.37 P.M.
3.37+
3.39
3.39+
3.40
3.40+
3.40+
3.40½
3.40½+
3.41
3.41+
4.55 P.M.
C
5.24 P.M.
5.26
5.29
5.30
5.33
5.35
5.39
5.40
5.41
5.42+
5.56
5.57
5.58
5.59
6. P.M.
6.02
D
E
F

PLATE XII

Explanation of Plate XII

Outline sketches to illustrate changes of shape in several different organisms, and in certain instances prominent structural features, observed in hanging drop preparations kept at from 41° to 42° C. in the warm chamber.

A. Different forms assumed by an organism in observations made on Nov. 7, 1919. This organism when first seen showed wave-like pseudopodia. After retraction of the pseudopodia the organism then showed merely slow change of form.

B. Changes of form in an organism observed on Nov. 15, 1919. There was great rapidity of movement with pseudopodia quickly extended and retracted at times from various portions of the surface. The organism eventually spread out in a very thin sheet against the cover glass and moved about rapidly, showing an irregular outline except for a certain period, when it assumed a slug-like form. No pulsation was observed. The nearest approach to this was a quick reversal of movement as indicated in the three sketches following that illustrating the form assumed at 3:41 p. m. This reversal was repeated several times.

C. An organism studied on Nov. 15, 1919. Regular pulsation was noted on first finding the organism about three hours after the preparation was made. The movement consisted of a rotary pulsation of the interior of the organism occurring at regular intervals. Pseudopodia continued to be actively protruded throughout the observation. As the organism rotated upon itself, activity was noted at one point in its surface where there was a slender projecting structure, probably a rudimentary flagellum. This was plainly seen whipping in the surface of the organism at 5:56 p. m., but is not shown in sketch.

D. An organism observed in fresh preparation on Dec. 3, 1919. An extranuclear granule with five radiating lines is visible. In this material no pulsating forms were observed, although the organisms showed active amoeboid motion for fifty-four hours.

E. A parasite followed in observations made on Nov. 7, 1919. First wave-like pseudopodia were observed and in a short time the movement of these became rhythmical and the organism commenced to rotate rapidly.

F. A small organism observed on preceding date of observation. This was first seen sending out rather sharp, wave-like pseudopodia. These traveled in one direction and the organism soon began to pulsate regularly and to rotate actively.

ON THE RESISTANCE OF ASCARIS EGGS

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For several years, in my study on the development of *Ascaris lumbricoides,* I have been testing the resistant power of ascarid eggs against various chemical media in which the eggs were cultured. As far as references go, there are few reports on this subject, notwithstanding it is very important for prevention of ascaris infection.

Method: In the earlier part of my investigation I collected the eggs from the patient's feces dissolved in water, by filtering and then by centrifuging. The collected eggs are treated with a reagent in which the eggs are to be cultured. In the later part, however, I put the fecal mass in the reagent for some time, longer or shorter according to the purpose of experiments, then the eggs were collected from the fecal solution of the reagent by filtering and centrifuging, and lastly were put in a culture dish of the same concentration of reagent as that in which the above treatment is performed.

Culture dishes were kept in the laboratory room in the summer months and in the incubator at 31° C. in the winter.

The vitality of eggs and embryos in each dish was examined at regular intervals of time. The distinction between dead or living eggs was decided partly by microscopical examination and partly by animal feeding experiments. Young or immature eggs were chiefly tested by microscopical observation. The vital power was observed in some cases by transferring the eggs from a reagent into a water culture to test the further development. Even in the same culture there are a great many individual differences in longevity as in other organisms. Thus in the following tables the word "dead" means that the majority of the eggs in the culture indicated are dead, while there are very few eggs alive; and the word "alive" shows a majority of living eggs. Hence it is very difficult to determine exactly the date on which the eggs die or still live in a reagent.

Experiments: During the time from August, 1917, to January, 1918, I carried on a great many experiments, the result of which I published briefly in a Japanese journal of medicine. That is not so important and valuable, for it is all covered by the results obtained in the recent experiments, the results of which are tabulated as follows:

TABLE 1.—AFTER KEEPING THE FECAL MASS IN EACH REAGENT FOR TEN DAYS FROM JANUARY 11 TO 21, 1919, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C.

Reagents	Day							
	13th	18th	28th	31st	38th	43d	52d	
0.5% Nitric acid	E appear	EH normal E alive	do	do	do	do	do	Alive
1% Hydrochloric acid	E alive	do	do	do	do	do	do	Alive
5% Hydrochloric acid	E alive	EH swollen E alive	EH absent present E alive	do	do	do	do	Alive
7% Sulphuric acid	EH absent present E appear	E alive	EH absent or thinned E alive	E alive	do	E shrink		Dead
7% Glacial acetic acid	EH swollen E alive	EH destroyed E alive	EH dest. or absent E alive	E do	do	do	do	Alive
10% Formalin	EH normal E alive	do	E alive	do	do	do	do	Alive
12.5% Formalin	E alive	do	dried up					

E, embryo, EH, albuminous coating.

This table shows that the eggs in 7 per cent. sulphuric acid develop into embryos, but sooner or later they all die. On the twenty-eighth day (February 18) a part of each culture was transferred into the water culture, the further development of which is stated in Table 6.

TABLE 2.—AFTER FOUR HOURS IMMERSION OF FECAL MASS IN EACH REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C., ON FERUARY 3

Reagents	Day							
	5th	11th	15th	18th	25th	36th	49th	
0.5% Carbolic acid	F no or rarely 2	EH normal F no	F 2–4 V appear	V	do	do	do	Dead
1% Nitric acid	EH destroyed or absent F many	E appear	EH absent E alive	E alive	do	do	do	Alive
10% Hydrochloric acid	EH normal F many	EH swollen E alive	do	E alive F vac- uol	E alive	do	do	Alive
10% Sulphuric acid	EH normal F many	do V appear	E appear	E shrunk	do	E V	do	Dead
10% Glacial acetic acid	EH absent F many	do E alive	E alive F many	do	E alive V appear	E V	do	Dead
15% Formalin	EH normal F many	do V	F V	do	E few	E V	do	Dead
20% Formalin	EH normal F 6–8	....	F 6–8 V	....	......	..	..	Dead

F, blastomere; V, vacuole.

The table shows that in 0.5 per cent. carbolic acid or 20 per cent. formalin, ascaris eggs are unable to develop into embryos; in 10 per cent. sulphuric acid or glacial acetic acid, or 15 per cent. formalin, the eggs develop into embryos, but sooner or later die.

TABLE 3.—AFTER FIVE HOURS IMMERSION OF THE FECAL MASS IN EACH REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 10

Reagents	Day							
	4th	8th	11th	18th	23d	36th	42d	
0.6% Carbolic acid	EH normal F no	do V	V	do	do	do	do	Dead
1% Corrosive sublimate	EH normal F 4-6	F many	do	E appear	E alive F many	do	do	Alive
1.5% Nitric acid	EH absent or swollen F 2-7	do E appear	E alive	do	do	do	do	Alive
12.5% Sulphuric acid	EH normal F 2-4-6	do E appear	E shrink	do	do	do	do	Dead
12.5% Glacial acetic acid	EH swollen or absent F 2-4-6	do E appear	E alive	do	E alive V appear	E V	do	Dead
15% Hydrochloric acid	EH normal F 1-4	EH ab, or present E appear	E alive F V	do	E shrunken V appear	do	do	Dead
20% Hydrochloric acid	EH normal F 4-7	EH absent or swollen E appear F V	E alive or shrunken	E shrink and V	do	do	do	Dead
25% Formalin	EH normal F no or 2-4	do	F V appear	F V	do	do	do	Dead
Human urine	EH normal F no	do	do	rarely F 2	do	V appear	V	Dead

From this table it is seen that in 0.6 per cent. carbolic acid, or in 25 per cent. formalin, ascaris eggs are unable to develop into embryos; in 12.5 per cent. sulphuric acid or glacial acetic acid, or in 15 per cent. and 20 per cent. hydrochloric acid, the eggs may develop into embryos, but die later; 1.5 per cent. nitric acid is not harmful to the development of the eggs.

TABLE 4.—AFTER FOUR HOURS IMMERSION OF FECAL MASS, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 14

Reagents	Day					
	5th	14th	19th	30th	43d	
0.1% Potassium permanganate	EH normal F no	rarely do F 2-4	E alive	do	do	Alive
0.5% Potassium permanganate	EH normal F no	rarely do F 2-4	E alive	do	do	Alive
1% Iron sulphate	EH normal F many	do E appear	E alive	do	do	Alive
5% Iron sulphate	EH normal F many	do E appear	E alive	do	do	Alive
10% Sulphuric acid	EH normal F many	do E appear	E alive or shrunken	E V	do	Dead
15% Hydrochloric acid	EH normal F many	slightly shrunken	shrunken	V	do	Dead
20% Hydrochloric acid	EH swollen destroy F many	F many V appear	V	do	do	Dead
1% Nitric acid	EH slightly swollen F many	do E appear	E alive	do	do	Alive

The embryos cultured in 0.5 per cent. solution of potassium permanganate emerged from the egg-shell alive. This is a most interesting fact in the study of ascaris development.

TABLE 5.—AFTER TWO DAYS IMMERSION OF FECAL MASS IN THE REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 20

Reagents	Day			
	8th	13th	37th	
0.02% Potassium permanganate	EH normal F no rarely 2-4	F many E appear	E alive	Alive
0.05% Potassium permanganate	EH normal F no rarely 2-4	E alive	do	Alive
10% Iron sulphate	EH normal E appear	F many E alive	E alive	Alive

TABLE 6.—AFTER TWENTY-EIGHT DAYS, THE EGGS WERE TRANSFERRED FROM THE REAGENT (TABLE 1) TO THE WATER CULTURE ON FEBRUARY 18

Date	Reagents				
	0.5% Nitric Acid	5% Hydrochloric Acid	7% Sulphuric Acid	7% Glacial Acetic Acid	10% Formalin
21st day	E alive	E alive	F many E appear	do	F many
34th day	E alive	do	do	do	F many E alive

TABLE 7.—AFTER FIFTEEN DAYS, THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE ON FEBRUARY 18 (See TABLE 2)

Date	Reagents						
	0.5% Carbolic Acid	1% Nitric Acid	10% Hydrochloric Acid	10% Sulphuric Acid	10% Glacial Acetic Acid	15% Formalin	20% Formalin
10th	F no V	E alive	E alive	F shrunk	F many E appear	F many V	V
15th	V	E alive	E alive	F shrunk V	E alive	F V	V
21st	V	E alive	E alive V appear	V	E alive	V	V
34th	V	E alive	V	V	E alive	E no F V	V
	Dead	Alive	Dead	Dead	Alive	Dead	Dead

This table shows that eggs cultured in 10 per cent. hydrochloric acid or sulphuric acid, and in 15 or 20 per cent. formalin died by the fifteenth day of cultivation.

TABLE 8.—AFTER EIGHT DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT, ON FEBRUARY 18 (SEE TABLE 3)

Date	Reagents								
	0.6% Carbolic Acid	1% Corrosive Sublimate	1.5% Nitric Acid	12.5% Sulphuric Acid	12.5% Glacial Acetic Acid	15% Hydrochloric Acid	20% Formalin	25% Formalin	Urine
10th	F no V	F many	F many E alive	F many	E appear alive	F many E appear	E alive or V	V	
15th	V	F many E ailve	E alive	....	E alive	E alive			
21st	V	E alive	E alive	F shrunk	E alive	E alive	E V	F V	
34th	V	E alive	E alive	shrunk	E alive	E alive	E shrunk	V	F no 2-4
	Dead	Alive	Alive	Dead	Alive	Alive	Dead	Dead	Alive

Eggs in 0.6 per cent. carbolic acid, in 12.5 per cent. sulphuric acid or in 20 per cent. and 25 per cent. formalin were so injured as to be unable to develop further by the eighth day.

TABLE 9.—AFTER FOURTEEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT IN THE LABORATORY ROOM, AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 28

Date	Reagents			
	0.1% Potassium Permanganate	0.5% Potassium Permanganate	15% Hydrochloric Acid	20% Hydrochloric Acid
11th day	F many E alive	F no rarely 2-7	shrunken	V
24th day	E alive	E alive	V	V
	Alive	Alive	Dead	Dead

The eggs in 15 or 20 per cent. hydrochloric acid could not develop by the fourteenth day.

TABLE 10.—AFTER TEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT CULTURE ON FEBRUARY 28

Date	Reagents			
	0.1% Potassium Permanganate	0.5% Potassium Permanganate	0.5% Carbolic Acid	0.6% Carbolic Acid
23d day	F no rarely 2-4	F no rarely 2-4	F no rarely 2	F no rarely 2
28th day	F many E alive	F many E alive	F no or 2	F no or 2
	Alive	Alive	Alive	Alive

Ascarid eggs retain the power to develop during ten days in 0.5 or 0.6 per cent. carbonic acid.

TABLE 11.—AFTER FOURTEEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT ON MARCH 11

Date	Reagents							
	7% Formalin	10% Formalin	15% Formalin	20% Formalin	10% Sulphuric Acid	12.5% Glacial Acetic Acid	15% Hydrochloric Acid	20% Hydrochloric Acid
13th	E alive	E alive	E alive	E alive	F	F many	EH absent E F	R alive
18th	do	do	do	do	E alive	E appear F many	E alive	do
	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive

All above experiments are summarized in a table as follows:

Potassium permanganate	0.02% alive	0.05% alive	0.1% alive	0.5% alive	
Corrosive sublimate	1% alive				
Nitric acid	0.5% alive	1% alive	1.5% alive		
Iron sulphate	1% alive	5% alive	10% alive		
Formalin	7% alive until 14th	10% alive	15% dead after 15-18	20% dead after 15-20	25% dead before 8th
Hydrochloric acid	1% alive	5% alive	10% alive	15% dead after 11-14	20% dead after 8, 11 or 14
Glacial acetic acid	7% alive	10% alive, but dead after after 25-30th	12.5% alive until 8th, dead after 28th		
Sulphuric acid	7% alive, dead after 43d	10% dead after 11-15	12.5% dead after 8-11th		
Carbolic acid	0.5% alive 8th, dead after after 11-15	0.6% alive until 10, dead after 8-11th day			
Human urine	Vacuoles appear after 36 or 42, and dead after 70th day				

K. Hotta, a student of our college, also made elaborate experiments on the same subject under my direction during the past year from June, 1918, to May, 1919. The results of his experiments coincide essentially with those of mine, with the exception of a slight difference.

The summarized tables are given as follows:

	Reagents				
	Hydrochloric Acid	Carbolic Acid	Sulphuric Acid	Formalin	Glacial Acetic Acid
Able to develop in	14%	0.3%	9%	12%	8%
Unable to develop in	15%	0.4%	10%	....	9%

Hydrochloric acid	15% alive until 12, 13	17% alive until 12, 11	19% alive until 10	25% alive until 6, 7	28% alive until 5th
Sulphuric acid	10% alive until 12th	12% alive until 11th	15% alive until 11th	20% alive one day	25% dead within day
Formalin	20% alive until 7, 8th	25% alive 7 days	27% alive 6 days		
Carbolic acid	0.4% alive until 30th	0.6% alive 11 days	0.8% alive 10 days		

It is a most important and interesting fact that ascarid eggs are unable to develop and ultimately die in human urine. From some experiments I have assumed that the urine acts more effectively upon eggs at a higher temperature (31° C.) than in the lower (10° C.) for destroying the power of their development.

The influence of the reagent on the ascarid eggs depends upon the permeability of the coverings of the egg. In the above experiments, for instance, formalin and sulphuric acid act to coagulate the albumin-coating of ascarid eggs in consequence of which the penetration of the fluid is prevented. After long action, glacial acetic acid and nitric acid destroy or break down the albuminous membrane of the eggs, but do not penetrate easily through the inner chitinous membrane. For these reasons eggs cultured in those reagents may resist a higher concentration and survive much longer. Hydrochloric acid will also do the same thing.

Carbolic acid, however, may penetrate the egg membrane more easily and more effectively than any other reagents which I have used in the above experiments. Urine contains several kinds of ferments by which the albuminous membrane of the egg may be dissolved. Action of the ferments seems to be accelerated by increasing the temperature as stated above. Moreover, the urine concentration is so much higher than that of the egg-content that the osmotic pressure is sufficiently great to facilitate introducing the urine into the egg-shell.

Besides these experiments on chemicals, I have made some other experiments on the resistance of ascaris eggs to cold.

Exp. 1.—Mature eggs cultured in the incubator from September 28, 1917, to December 11, were put under ground and covered by a thin lawer of soil, and on May 2, 1918, the eggs were given to a guinea-pig which was surely infected.

Exp. 2.—Mature eggs cultured in the incubator from October 27, 1917 to December 22, were put on the ground out-doors, and on May 2, 1918, they were given to a guinea-pig which was also evidently infected.

Exp. 3.—Immature eggs collected on December 8, 1917, were put on the ground from December 13, 1917, to May 2, 1918, then they were transferred to the incubator and kept again in the laboratory room from June to October; next they were put in the incubator during November and December, and in the laboratory room from January to February, 1919, then finally in the incubator from March 1 to 19. On the last date the eggs were given to two guinea-pigs which were killed after a week and showed an infection.

A NEW BI-FLAGELLATED PROTOZOON OF MAN

Toynbee Wight
Captain, M. C., U. S. Army
AND
Baldwin Lucké
First Lieut., M. C., U. S. Army
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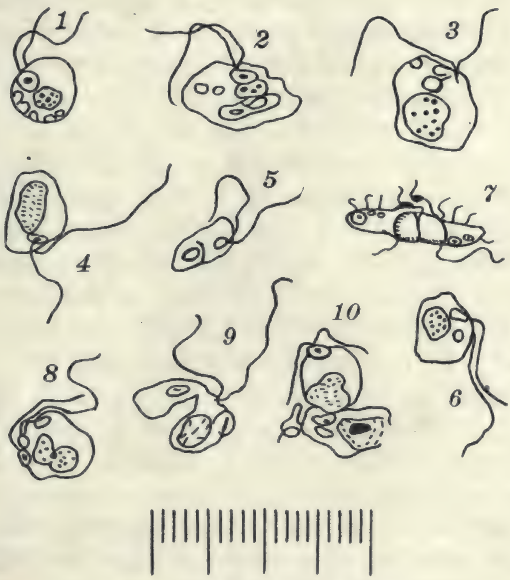
In the study of postmortem cultures and direct smears from various organs of soldiers, we have found what appears to be a hitherto undescribed flagellate protozoon. This organism has been observed in three individuals, occurring respectively in the lung, the sphenoidal sinus, and the heart's blood. Its discovery, in the first place, was more or less accidental. It has been the custom here to supplement bacterial cultures with direct smears from the organs examined in order to study phagocytosis and other cellular phenomena. These smears were fixed in methyl alcohol, stained by Gram's method and counterstained in a weak, watery solution of eosin. Through an oversight a smear from the heart's blood of an influenza patient was left in the eosin solution for several hours; on examining the slide there were found flagellated organisms, and on examining the blood-agar plate culture of the heart's blood, similar structures were observed. Within a few days like protozoa were found in two new autopsies, both in direct smears and in the cultures from the sphenoidal sinus and the lung. Unfortunately the pressure of work during the epidemic of influenza did not permit us to investigate the flagellate closely at that time.

The original smears and slides made from the cultures were preserved after fixation in methyl alcohol; sublimate alcohol fixed slides were lost. Various stains were employed such as Giemsa's, Jenner's, eosin, methylene blue and eosin and iron hematoxylin. It was found that best results were obtained with weak watery solutions of eosin, staining over night. While the Giemsa preparations showed more clearly the internal structures, the flagella were generally so weakly stained that it was difficult to recognize them. It can be seen that our material was not fixed or stained by the best methods, and we are therefore unable to describe in detail the internal structures of the organisms. We realize that our studies are of necessity fragmentary and inconclusive, but we desire to record the findings in order to stimulate search for similar organisms.

MORPHOLOGY

The best preserved organisms were round or pear shaped and measured on an average of 6.5μ in their longest diameter, and 5μ in their greatest width. The extremes of measurements were 6 and 11μ for length, and 3 and 6μ for width. Near the more pointed end could be seen a small kinetonucleus, usually surrounded by a clear area. Sometimes two basal granules whence two flagella took their origin could be recognized near the kinetonucleus; it was a rule for them to have a distinct origin and to be well separated from one another at this



EXPLANATION OF FIGURES

Camera lucida drawings of various forms of the organism described. The small divisions on the scale are each one micron.

Fig. 1 to Fig. 6.—Typical organisms are shown possessing vacuoles, kinetonucleus, trophonucleus with chromatin granules or rods, and flagella.

Fig. 7.—Shows apparently division of trophonucleus. Adherent to the periphery of the protozoon are bacteria-like structures of unknown nature.

Fig. 8.—The trophonucleus is well divided.

Fig. 9.—Shows almost complete cell division.

Fig. 10.—Shows two adult protozoa adherent to one another.

point. These flagella were free, sometimes of equal and other times of unequal length, the shorter averaging 8μ, the longer 14μ. Toward the distal or more rounded end there was a large, trophonucleus, round or oval in shape and averaging 3.5μ in diameter. Coarse chromatin granules or rods could frequently be recognized; in several instances these appeared near the periphery of the nucleus in the form of a dis-

tinct ring. A small, well marked karyosome could often be seen within the nucleus. Within the cytoplasm, were generally several vacuoles; whether these be of contractile nature or food vacuoles we were unable to determine. The outline of the organisms was usually clear and distinct, but adherent to the periphery of several of the specimens were what appeared to be large bacteria.

Several examples were found which seemed to throw some light on reproduction. Thus, we have seen organisms with apparent division of their nuclear structures; others with a deep constriction in their body; and occasionally two fully formed protozoa were adherent to one another. It would seem then that these flagellates divide by binary fission. We also encountered small round bodies, averaging 4μ in diameter, showing the general internal structure, but no indication of flagella; these were looked upon as possible cystic stages.

Living forms were easily observed in cultures on rabbit's blood glycerine agar, on which we were able to cultivate the protozoa to the fourth generation at room temperature. These cultures were plentiful, always in association with various bacteria, chiefly streptococci and pneumococci. The organisms were actively motile, but the use of their flagella was not studied in sufficient detail. In cultures, but never in direct smears from the autopsy material, two other noteworthy structures were observed. One was like a large bacillus, the other like the head of a spermatozoon with a deeply staining granule. Both of these had a well stained yet delicate single flagellum, four to six times as long as their body. These structures might be connected with the genesis of the protozoon, but such a statement necessarily leads into speculation. Sure it is that none of the flagella of bacteria stained with dilute aqueous eosin; many of these bodies resembled the structures adherent to the wall of the protozoa.

DISCUSSION

The organisms described above occurred in every instance in cases of acute influenza. Careful analysis of the history of these patients and of the anatomical changes discovered at the autopsy brings out no additional information. The three patients seemed to have run a disease course exactly similar to that of other influenza patients of that period. As yet we have been unable to find the protozoa in the histologic sections from these cases and we have observed no microscopic tissue changes differing from those of other influenza patients. Klebs has described minute monads and attributed to them some rôle in the pathology of influenza. His observations have, however, not been confirmed. Since we have only found these protozoa in three bodies of 126 influenza necropsies studied in detail, and since the tissue of

these cases presented no unusual alterations, we would look upon the organisms as accidental invaders, possibly from the oral cavity. It is easily seen how such protozoa could make their way from the mouth cavity to the respiratory tract; their presence in the heart's blood can be explained by postmortem invasion from the lungs through the pulmonary veins.

Various flagellated protozoa have been described as occasionally inhabiting the oral cavity or the lungs. Thus, Fantham, Stephens and Theobald state "Prowazek speaks of a variety of *Trichomonas intestinalis* inhabiting the oral cavity. This was distinguished by a posterior process exceeding the length of the body fourfold, and by a somewhat unusual course of the undulating membrane. The food of this form, which was found in the whitish deposit present, especially in the cavities of carious teeth, consisted almost exclusively of micrococci. Schmidt and St. Artault named the Trichomonads found in pathological products (e. g., gangrene, putrid bronchitis, phthisis) of the lungs of man, as *Trichomonas pulmonalis*. Trichomonads have also been found by Wieting in lobular pneumonia in the lungs of pigs."

It is difficult to assign the protozoa which we have described to a definite place because of the insufficient data we possess. The fact that these organisms appear to constantly possess two flagella, places them in the family of Bodonidae, Bütschli, while the possession of a kinetonucleus would place them in the genus Prowazekia, Hartmann and Chagas, 1910. This, however, is only a tentative assignment.

SUMMARY

A small biflagellated protozoon was found in direct smears and in cultures from three postmortems of patients dead from acute influenza. They occurred, respectively, in the heart's blood, sphenoidal sinus and the lung, and apparently produced no tissue changes. The organisms were round or pear shaped, possessed two free flagella and a kinetonucleus. They were easily cultivated on rabbit's blood glycerin agar. We regard these organisms as accidental invaders, possibly belonging to the genus Prowazekia.

ARTICLES CITED

Fantham, H. B., Stephens, J. W. W., and Theobald, F. V. 1916.—The Animal Parasites of Man. William Wood, New York.

Klebs, E. 1890.—Weiteres über Influenza. D. med. Wchnschr., 16: 278-280.
1890.—Ein Blutbefund bei Influenza. Centralbl. f. Bakt. u. Paras., 7: 145-151.

QUELQUES OBSERVATIONS SUR LES PÉDICULIDES

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A l'occasion du typhus exanthématique, qui a sévi au milieu de l'armée et de la population roumaine pendant la terrible guerre, en 1917, j'ai pu faire les observations suivantes sur le pou de corps (*Pediculus vestimenti*).

1. *Les substances recommandées par Prowazek, Versluys, et d'autres, sont inefficaces.*—Aussitôt que le typhus exanthématique a commencé à se répandre parmi la population de la ville de Jassy, j'ai publié un opuscule (1917) destiné à vulgariser l'emploi des diverses substances, que les auteurs recommandaient contre les poux. Déjà les pharmacies mettaient en vente des fioles contenant diverses espèces d'huiles essentielles.

Une fabrique locale de savon, "Carmen Sylva," fabriquait elle aussi un spécifique lancé sous le nom séduisant d'*Exantol.* De leur côté les journaux recommandaient différentes compositions préparées avec des huiles essentielles.

J'ai étudié et expérimenté moi-meme l'action de ces diverses substances sur les poux, afin de constater si, vraiment, l'odeur ou les propriétés chimiques de ces substances avaient pour effet de les éloigner. En 1917, à cause que les poux étaient exanthématiques, je fus obligé de borner mes expériences exclusivement "in vitro." Aujourd'hui (1919), aucun cas de typhus exanthématique n'existant plus à Jassy, j'ai expérimenté la manière du comportement de ces parasites sur mon propre bras. J'ai commencé par faire des expériences avec les substances recommandées par Prowazek: *essences d'eucalyptus, de clous de girofle d'anis.* J'ai pris trois cristallisoirs; dans chacun d'eux j'ai placé 5 poux (*Pediculus vestimenti*) sur un morceau de flanelle. Sur la flanelle d'un des cristallisoirs j'ai fait tomber des gouttes d'essence d'eucalyptus; sur le second des gouttes d'essence de clous de girofle; sur le troisième des gouttes d'essence d'anis. Les poux, non seulement ont continué à vivre de 12 à 24 heures, mais les femelles pendant ce temps ont même pondu leurs lentes. Eysell recommandait en 1915, de saupoudrer la peau avec soufre pilé. J'ai saupoudré mon propre bras et j'y ai placé dessus un pou famélique qui, malgré le soufre, me piqua et me suça. De la même manière se sont comportés d'autres poux, quand j'ai fait des expériences avec de l'essence de térébenthine, recommandée par Marschalkó (1915), avec du baume du Pérou, recommandé par Meltzer (1915), avec de la teinture d'acorus calamus,

recommandée par Versluys (1915) ; ce qui prouve qu'aucune de ces substances n'est d'aucune efficacité contre les piqûres des poux.

2. *Les poux de corps sucent aussi d'autres animaux.*—Galli-Valerio a fait des expériences avec des poux de tête (*Pediculus capitis*), et il a pu démontrer qu'ils sucent aussi d'autres animaux: les cobayes et les souris blanches. Nous avons fait les mêmes expériences avec des poux de corps (*Pediculus vestimenti*). Sur cinq poux que nous avons placés sur un chien, trois ont sucé; sur quatre placés sur un chat, deux ont sucé; sur cinq mis sur un lapin, un seul a sucé.

Nous n'avons pas réussi à les faire sucer sur des grenouilles, sur des poules ni sur des pigeons. En tout cas quand on veut faire ces expériences il faut choisir des poux faméliques.

3. *Action des substances grasses.*—Les bergers roumains restent, pendant de longs mois, à garder leurs troupeaux à la montagne, sans jamais changer de linge, et cependant ils n'ont jamais de poux sur leur corps. C'est qu'ils imprègnent leurs chemises et aussi leurs pantalons de laine blanche, qui se trouvent en contact direct avec la peau, de petit lait ou de beurre fondu; après avoir tordu ces vêtements, pour en exprimer la partie liquide, ils s'en habillent et sont sûrs d' immunité.

J'ai recherché quelle est l'action du beurre sur les poux. Dans ce but j'ai étendu un morceau de flanelle, imprégné de beurre fondu, dans un cristallisoir, et sur cette flanelle j'ai placé un pou femelle (*P. vestimenti*) qui n'avait pas encore pondu les œufs, dont elle était pleine. Dans un autre cristallisoir j'ai disposé un autre morceau de flanelle, non imprégnée de beurre fondu, et sur elle aussi j'ai placé une femelle avant sa ponte. Qu'est-il arrivé? La femelle placée sur ce dernier morceau de flanelle non graissée a pondu et agglutiné chacune de ses lentes d'une manière régulière le long des fils effilochés du tissu; tandis que celle placée sur le morceau de flanelle imprégnée de beurre a déposé ses œufs sur les fils effilochés sans les y coller. J'ai répété les mêmes expériences avec de l'huile d'olive, de la vaseline, du pétrole, et toujours j'ai constaté que toutes ces substances grasses empêchent le collage des lentes sur les fils du tissu, dont sont faits les vêtements qui en sont imprégnés.

En outre, les substances grasses, en collant les opercules des lentes, tuent les larves, asphyxiées avant l'éclosion; et les adultes aussi périssent, après quelque temps, les orifices des organes de la respiration restant obstrués par ces mêmes matières.

Parmi toutes les substances grasses, celle qui se trouvait chez nous en plus grande abondance étant le pétrole, je n'ai pas cessé de le recommander à l'occasion de la guerre.

4. *Variétés des poux de corps.*—Souvent il m'est arrivé de recevoir, de la part des médecins d'hôpitaux d'exanthématiques, des échantillons

de poux rencontrés sur le corps des malades, avec prière de les examiner et de leur communiquer s'il est possible d'en distinguer des variétés diverses. J'ai reçu aussi des poux des différents camps de concentration des prisonniers bulgares, hongrois, turcs et allemands.

Ce qui faisait soupçonner à ces jeunes médecins la possibilité de l'existence de plusieurs variétés de poux de corps, c'était l'extrême différence de couleur, de taille, de mouvement que l'on remarquait entre eux, et surtout leurs antennes qui, chez les uns étaient constituées de trois articles et chez d'autres de cinq.

Les poux de corps quand ils sont jeunes ont une couleur jaune-verdâtre; ils peuvent devenir blanchâtres, jusqu'au blond-châtain. Au moment où ils sucent du sang ils deviennent rouges, mais seulement beaucoup plus tard ils deviennent noirs. Cette couleur noire peut avoir deux causes: ou que le sang du tube digestif, après un certain temps, devient noir, et le corps, à cause de sa transparence, paraêt être de la même couleur; ou que le tégument lui-même devient noir.

La taille du pou varie du moment qu'il a quitté l'œuf jusqu'à ce qu'il devient adulte; mais jamais la longueur du mâle ne dépasse les 3 mm. et celle de la femelle les 4 mm.

La vitesse du mouvement chez les poux dépend d'une foule de circonstances. Les poux faméliques cherchent la lumière, tandis que ceux rassasiés, évitent la lumière. Cela explique pourquoi le matin on en trouve sur les vêtements (surtout sur le col) une moins grande quantité que le soir.

Pour ce qui a rapport à la différence des antennes: les poux à trois articles étaient des larves, et ceux à cinq articles étaient des adultes.

De sorte qu'on peut affirmer qu'il existe une seule espèce de pou de corps (*Pediculus vestimenti* Nitzsch-*Pediculus corporis* de Geer) sans aucune variété; espèce qui se distingue seulement de celle du pou de tête (*Pediculus capitis* de Geer).

5. *Les mouches comme agents de transmission des poux.*—J'ai répété l'expérience de Galli-Valerio, en plaçant sous une cloche en verre deux mouches (*Musca domestica*) et un morceau de flanelle sur laquelle étaient déposés plusieurs poux. J'ai saupoudré l'étoffe avec du sucre pour attirer les mouches près des poux. Après 24 heures j'ai trouvé fixé au thorax de l'une d'elles un pou. Elle voletait de-ci de-la sans que le pou tombât. J'ai enlevé les ailes de la mouche et puis je l'ai laissée se promener sur mon bras gauche mis à nu. Le pou, après une quinzaine de minutes s'est détaché du thorax de la mouche en tombant sur la peau de mon bras.

Cette expérience nous paraît suffisante pour nous faire admettre que les mouches peuvent très bien servir de véhicule aux poux.

6 *Le typhus exanthématique ne peut se propager par la poussière.* — Le Dr. Imbert qui jadis fut membre de la Mission francaise d'épidémiologie en Serbie, et qui en 1917 était détaché chez nous, a publié dans les journaux locaux un article dans lequel il exprimait l'opinion que le typhus exanthématique peut se propager par la poussière provenant de la décomposition des poux exanthématiques. Les poux morts—disait-il—et les débris mêmes des poux morts qui se trouvent sur le sol, et qui contiennent encore le virus contagieux, sont émiettés. desséches, pulvérises et puis soulevés en l'air, soit par le balayage, soit par les traînes des robes, soit simplement par la démarche, après quoi ils pénètrent par les narines et par la bouche et sont inspirés.

Eh bien! parmis les nombreux hôpitaux d'exanthématiques installés à Jassy, il y en avait quelques-uns qui se trouvaient précisément au centre de la ville. Un grand nombre de fenêtres de ces hôpitaux s'ouvrent directement sur les rues. Incessamment, surtout au printemps, par une insouciance déplorable, on balayait les salles, les fenêtres extérieures étant grandes-ouvertes, et on secouait dehors la literie des malades; de sorte que la poussière tombait directement sur le trottoir de la rue, partant sur les passants.

Et cependant il n'y eut d'atteints de la terrible contagion que ceux qui, se trouvant en contact direct ou indirect avec les malades, emportaient sur leurs personnes des poux vivants.

References Cited

Eysell, T. 1915.—Ein einfaches Vorbeugungsmittel gegen Verlausung und ihre Folgen. Münch. med. Wchnschr., 62: 351-564.

Galli-Valerio, B. 1915.—Erfahrung über den Schutz gegen den Läusestich. Centralbl. Bakt. Par., I. Orig., 77: 262-264.

1916.—Neue Beiträge zur Biologie und zur Bekämpfung der Läuse. Centralbl. Bakt. Par., I. Orig., 78.

Imbert. 1917.—Tifosul exantematic, despre propagarea boalei (en roumain). Miscarea no. 63.

Léon, N. 1917.—Primejdia insectelor in imp de razboin si mijloacele a le combate. Tipografia Goldner, Jasi, 3. Martie.

Marschalko, Th. von. 1915.—Die Bekämpfung der Läuseplage im Felde. D. med. Wchnschr., 41: 316-317.

Meltzer, Otto. 1915.—Die Bekampfung der Läuseplage im Felde. D. med. Wchnschr., 41: 532-533.

Prowazek, S. von. 1915.—Bemerkungen über die Biologie und Bekämpfung der Kleiderlaus. Münch. med. Wchnschr., 62: 67-68.

Versluys, T. 1915.—Ueber die Verbreitung von Seuchen durch Insekten im Kriege. Leipsig.

ON A NEW SPECIES OF RHABDITOID WORMS FOUND IN THE HUMAN INTESTINES

Harujiro Kobayashi

Most species of Rhabditis and its allies are found free living in the earth and decayed vegetable matter. Several examples of parasitic Rhabditis, however, have been reported, e. g., *Rhabditis pellio* (Scheiber) in the urine (Scheiber 1880, Baginsky 1887), *Rhabditis* niellyi (Blanchard) in the skin (Nielly 1882), *Rhabditis* sp. in the stomach (Frese 1907) and others. Such were also found parasitic in certain other species of mammals. They may be true parasites, but most of them seem rather to be facultative parasites.

In 1914, when I was engaged in the microscopical examination of the feces of the pupils of a primary school in Ibaraki Prefecture, Japan, for the eggs of parasites, I found several times in the fresh feces worms belonging to a certain species of Rhabditis. It seemed to be new to science and to it I gave the name *Rhabditis hominis* n. sp.; it is this with which I am going to deal in the present communication.

Rhabditis hominis is viviparous and found numerously in the freshly passed feces (strict precautions were taken to exclude free living nematodes). All the stages of its development occurred in the same specimen of feces, i. e., the adults and young of both males and females and the newly hatched larvae.

A full grown female measures 1.5 to 2 mm. in length and 0.12 mm. in breadth. In the cuticula fine transverse striations are seen. The body has a cylindrical shape of nearly uniform diameter, altho it tapers gradually anteriorly from the part of the esophagus. Posteriorly, the body narrows more abruptly at the region of the anus and becomes a long and fine tip. The tail (from the level of the anus to the end) measures 0.17 to 0.24 mm. in length. The oral orifice is surrounded by four labial palpi, and leads to the oral cavity consisting of a relatively long canal, measuring 0.02 to 0.04 mm. in length. The muscular esophagus has a length of 0.17 to 0.2 mm. It consists of four parts: (1) a relatively long and broad anterior canal; (2) the anterior bulbus; (3) a narrower posterior canal, and (4) the posterior bulbus. The anterior bulbus lies at the middle of the whole length of the esophagus and the oral cavity. The anterior canal region is slightly shorter than the posterior one. The posterior bulbus is 0.02 mm. in diameter and smaller than the anterior. The internal lumen

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of the esophagus gradually narrows posteriorly. The broadest part of it at the anterior end of the anterior canal is 6μ in width, being the same as that of the oral cavity, while its posterior half is apparently closed. It again widens slightly in the anterior bulbus and in the posterior canal region it again closes. The intestine consists of two rows of cells. The two ends of the intestine are thicker than the middle, which occupies the largest part and is compressed by the genital organs. Near the posterior end the intestine narrows abruptly and is connected to the rectum. The excretory canal opens at the level of the posterior bulb of the esophagus.

The worm has a vulva, which opens at about the middle of the body, and paired ovaries and uteri. The ovaries consist of club-shaped rows of cells. The anterior ovary arises slightly anterior to the anus and runs anteriad. Just in front of the middle of the body (or near the posterior end of the esophagus) it narrows and is connected with the uterus. The uterus runs farther anteriad and near the posterior bulb of the esophagus turns posteriad to join the vulva. The posterior ovary arises slightly posterior to the esophagus (almost at the same level as the bend of the anterior uterus) and is continued posteriorly to the uterus, which runs further posteriad. Before it reaches the posterior end of the intestine, it turns anteriad and ends in the vulva. In somewhat younger specimens, the turning point of the posterior uterus and the anus are about 0.16 mm. apart. In the young specimens, each uterus is filled with 10 to 50 eggs, the larger specimens carrying more than the smaller. The posterior uterus is somewhat shorter than the anterior, if not the same length. The uteri of a full grown worm are filled witth hatched embryos. The eggs in the uteri are somewhat irregularly ellipsoidal and measure 44 to 28 by 28 to 32μ.

Full grown males measure 0.9 to 1.2 mm. in length and 0.03 to 0.05 mm. in breadth. They are similar in form to the females. The posterior part narrows abruptly at the region of the bursa copulatrix and forms the tail which curves somewhat laterad. The anus and the posterior end of the body stand 0.064 to 0.07 mm. apart. The tail is 28μ long. The alimentary canal is similar in structure to that of the female, but the esophagus of the male is shorter and smaller than that of the female, measuring 0.13 to 0.14 mm. in length. The male has one testis, which arises directly posterior to the esophagus and runs posteriad, forming the vesicula seminalis. It has two spicules of the same size, 35 to 41μ long. The terminal part of each spicule curves exteriorly and ventrally, and has the shape of a sickle. Dorsally to the spicules, a rod-shaped gubernaculum is present.

A bursa copulatrix is present. The anterior end of this organ lies at about the same level as the anterior end of the spicules and the

posterior end reaches to the place where the body narrows abruptly. It is narrow and is provided with six pairs of stalked papillae; two of them are preanal, while the third pair lies almost at the same level as the anus. The first and the second pairs, and the third and the fourth pairs lie closer together than the second to the third, and the fourth to the fifth pairs. The fifth and the sixth pairs lie closest together of all.

The youngest larvae measure 0.24 to 0.3 mm. long by 0.012 to 0.03 mm. wide. They have an esophagus, which is relatively long, occupying about one third of the entire body length; it contains two bulbi. The oral cavity can be clearly recognized.

The worms were found parasitic in the pupils of the primary schools in the counties of Soma and Inashiki. Seventeen cases out of 668 examined (i. e., 2.5 per cent.) were infected. All the cases were boys and girls between 10 and 14. The examination was carried out during October and November. The worms were found in considerable numbers. Furthermore, Dr. O. Takaki found 3 cases out of 471 pupiles examined in the primary schools of the county of Kitasoma. His examination was made in June, 1914.

It is interesting to note that four pupils out of the seventeen who had been proved to harbor the worms by fecal examination in October and November, 1913, were all found free from the worm on their re-examination in January, 1914. In other words, the worms had entirely passed out of the body without any medical treatment in the course of two to three months. This fact shows that the worm may not be a true parasite, but happened to find a temporary or accidental lodgment in the human body. Still it seems to be highly probable that the worms can thrive in the human alimentary canal, for they were found abundantly and in all the stages of their development. The parasitologic importance of the species is to be studied in the future.

This nematode occurs in the larval and adult forms in the human feces, but no eggs are found in the feces, and therefore it should never be mistaken for *Strongyloides stercoralis* Bavay. Several Japanese articles regarding the discovery of *Strongyloides stercoralis* that were published some time back seem to have been dealing with this worm, especially those of Iwaya, Hasegawa and Chikada, and Shiga (see Japanese references below).

Dr. Takaki reports that these nematodes seem to have no pathogenicity towards their hosts, for the latter endure the presence of innumerable worms without the least sign of ill health.

Fig. 4

Fig. 1

Fig. 2

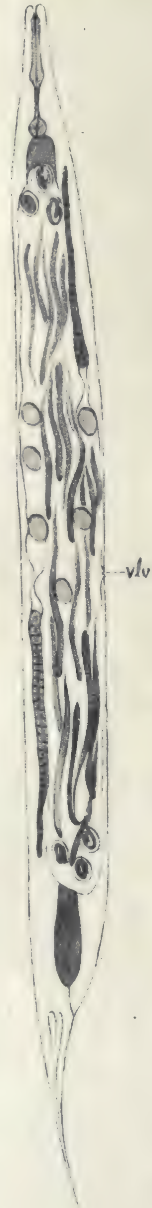


Fig. 3

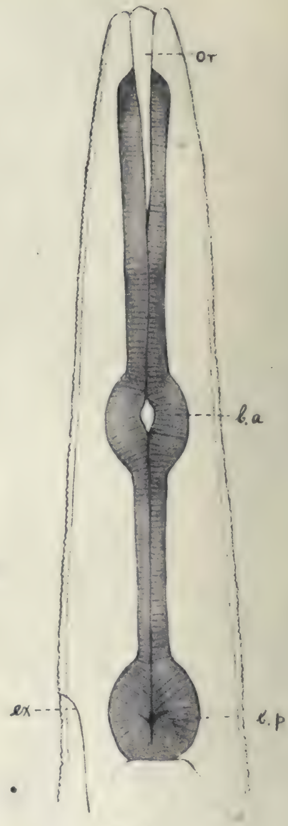


Fig. 5

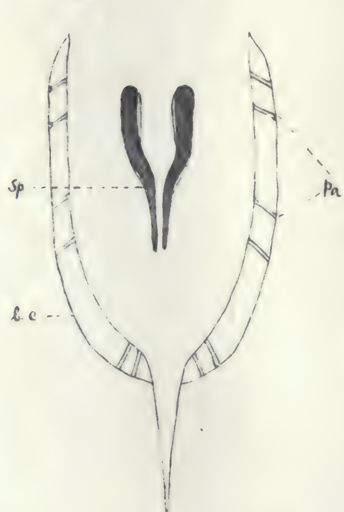
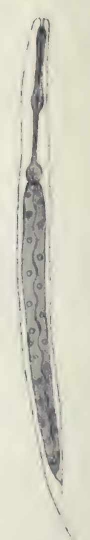


Fig. 6



H. Kobayashi del.

Literature Cited

Baginsky, A. 1887.—Hämoglobinurie mit Auftreten von Rabditiden im Urin. Deutsche med. Wchnschr., 13 : 60A.

Frese, O. 1907.—Ueber mikroskopische Würmer im Magen einer Ozaenakranken. Münch. med. Wchnschr., No. 11.

Nielly, N. 1882.—Un cas de dermatose parasitaire non encore observée en France (Anguillula leptodera?). Bull. acad. med., 46 : 395-402.

Scheiber, S. H. 1880.—Ein Fall von mikroskopisch kleinen Rundwürmern—Rhabditis genitalis—im Urin einer Kranken. Arch. path. anat., 82 : 161-175.

Japanese References

Iwaya, Shinnosuke.—On Anguillula stercoralis (Japanese). Tokyo-Ijishinshi, No. 1873.

Hasegawa, Tota, and Chikada, Ikuhei.—On Anguillula stercoralis obtained from the human body (Japanese). Ditto, No. 1584.

Shiga, Jutaro.—On a case of stenosis of the pulmonary arteries and Anguillula stercolaris (Japanese). Rikugun-Gunidan-Zasshi, No. 15.

Explanation of Plate XIII

Fig. 1.—Young female. × ca. 100.

Fig. 2.—Full grown female. × ca. 100.

Fig. 3.—Ditto; the oesophagus. × ca. 50.

Fig. 4.—Full grown male. × ca. 100.

Fig. 5.—Ditto; the posterior end. × ca. 500.

Fig. 6.—Larva. × ca. 300.

Reference Letters

a, annus; *ba*, anterior bulbus; *bp*, posterior bulbus; *bc*, bursa copulatrix; *ex*, terminal part of the excretory duct; *int*, intestine; *oes*, esophagus; *ov a*, anterior ovary; *ov p*, posterior ovary; *or*, oral cavity; *sp*, spicule; *t*, testis; *vlv*, vulva; *u*, uterus.

SPIROCHAETA RECURRENTIS: A FILTER PASSER

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Spirochaetes in an infective form can be forced through a Berkefeld filter by pressures of 50 pounds, and over, to the square inch (Todd and Wolbach, 1914). This note records an endeavor to ascertain the form in which *Spirochaeta recurrentis* passes through the filter.

Wolbach (1915) has shown, by sections, that *Spirochaeta elusa* is present everywhere in the walls of a Berkefeld filter through which these organisms have passed. Nine attempts were made to see *Spirochaeta recurrentis* in Berkefeld filtrates. Since all the experiments were of the same character, the description of one describes all.

(Experiment 595). Two, or more, rats were chloroformed. Heart blood was pipetted off. Organs were ground up with sharp sand in a 1 per cent. solution of sodium citrate in normal saline. The blood, with enough citrate solution to prevent coagulation and the ground-up organs, was passed through a well-impacted Buchner filter in order to remove red cells and organ débris. To prove the presence of spirochaetes in the Buchner filtrate specimens were examined and, to prove the infectivity of the spirochaetes seen, rats were inoculated. The growth of *Bacillus prodigiosus* from a four-day-old culture was then washed into 5 ccm. of normal saline and added to the Buchner filtrate. Control tubes, in which *B. prodigiosus* grew invariably, were inoculated from the filtrate. The Buchner filtrate was then passed through a Berkefeld filter under pressure varying from 50 to 90 pounds. To prove that the filters were intact culture tubes were inoculated with the filtrate; *B. prodigiosus* grew in none. To prove the infectivity of the Berkefeld filtrate rats were inoculated with it. To ascertain the form in which spirochetes existed in the filtrate it was examined.

In seven experiments, rats inoculated with Berkefeld filtrate became infected (Todd and Wolbach, 1914); in only two, of these seven, experiments were spirochaetes seen in the Berkefeld filtrate. In all nine experiments, infective material came from rats which were at the height of a first attack by one of four strains of *Spirochaeta recurrentis*. Each of the strains was known to produce a marked infection in white rats. The filters employed were either "W" or "N" Berkefeld filters. "W" filters were used for both experiments in which spirochaetes were seen in the filtrate.

There are two methods of proving the presence of spirochaetes. The first is the inoculation and demonstrated infection of a susceptible animal. The second is the detection of the parasites by microscopical examination. Both methods are fallacious. Each may reveal an infection where the other fails to do so.

The inoculation of susceptible animals is not an infallible test for the presence of spirochaetes. In these experiments, the strains

employed usually produced heavy infection in white rats, with many spirochaetes in the blood; young white rats were used since they are more susceptible to infection than are older ones. Yet, a few rats, shown to be susceptible by subsequent re-inoculation with the same strain, resisted inoculation by material in which spirochaetes were shown to be present and infective. Spirochaetes were present in resistant rats not at all or in numbers too small to be detected by the microscopical examination of blood. In some instances, spirochaetes have been shown to be present in a resistant rat by aspirating, under chloroform, blood from its heart and by inoculating and infecting with it a fresh rat.

Even repeated microscopical examination may fail to reveal spirochaetes in material known to contain them. As a rule, blood is examined for spirochaetes in thick films, dehemoglobinized and stained by some modification of Romanowsky's method. In order to compare this method with the examination of fresh preparations by dark ground illumination, a series of sixty-nine observations was made in which blood that might easily contain spirochaetes was examined simultaneously by the thick film and dark stage methods. There is little to chose between them. Twenty-six examinations were positive by the dark stage method and twenty-five by the thick film; four times spirochaetes were detected by the dark stage method alone, and thrice spirochaetes were found in thick films when they were unseen by dark stage examination. Thin preparations of blood, covered with ¾-inch square coverslips and ringed with vaseline were used for the dark stage examinations. They were always examined soon after they were made. Ten minutes were spent in the examination of each specimen, whether stained or fresh, before a negative examination was recorded.

Spirochaetes are not thrown down by centrifugalization as are trypanosomes (Dutton and Todd, 1905). Yet, spirochaetes may be seen in films of the precipitate thrown down by centrifugalization of fluids (coxal fluid from ticks, blood) in which previous examination failed to reveal them. Spirochaetes were found in precipitates obtained by centrifugalization at slow speeds, 200 to 500 revolutions per minute, for twenty minutes as well as in fluids centrifugalized at higher speeds, 2,000 to 3,000, for long periods, 90 to 240 minutes. Centrifugalization at low speeds was done in a small centrifuge, distance from the center to the bottom of the tubes being 14 cm. Centrifugalization at high speeds was done in a large centrifuge, the distance from the center to the bottom of the tubes being 24 cm. Ordinary urine centrifuge tubes holding 10 c.cm. were usually employed. It is of advantage to centrifugalize in two stages. Fluid is taken from the bottom of the

tubes first centrifugalized and placed for the second centrifugalization in smaller tubes each about 6 mm. in diameter and 13 cm. in length.

In order to ascertain whether all infective spirochaetes can be brought to the bottom of centrifuge tubes by centrifugalization, infective material was centrifugalized at high speeds, from 1,400 to 3,500 revolutions per minute, for periods ranging from twenty minutes to four hours. The top one-fifth or one-third of the fiuid was drawn off with a syringe, so soon as the centrifuge stopped, and injected into rats. In six out of nine experiments rats so inoculated became infected. In seven of these experiments the precipitate was searched for spirochaetes; they were found in three instances. Twice, the spirochaetes seen in the precipitate from fluids centrifugalized at high speeds were broken.

There was no conspicuous peculiarity in the morphology of the spirochaetes twice found in precipitate from centrifugalized Berkefeld filtrates. There were a few small forms, which would have been seen with difficulty in fresh preparations without dark field illumination; but, on the whole, the parasites were, if anything, rather larger than usual.

SUMMARY

1. *Spirochaeta recurrentis* can be forced in its type form through a "W" Berkefeld filter.

2. Centrifugalization, at the speeds and for the times employed, does not throw down all infective forms of *Spirochaeta recurrentis*.

References

Todd, J. L., and Wolbach, S. B. 1914.—Concerning the Filterability of *Spirochaeta duttoni*. Jour. Med. Research, 30: 27-36.

Dutton, J. E., and Todd, J. L. 1905.—The Nature of Human Tick Fever in the Eastern Part of the Congo Free State. Liverpool School Trop. Med., Mem. 17.

Wolbach, S. B. 1915.—On the Filterability and Biology of Spirochaetes. American Jour. Trop. Dis. Prev. Med., 2: 494-505.

VARIATION OF THE OVUM (*SARCOPTES SCABIEI*) UNDER COVERGLASS PRESSURE *

Fred D. Weidman

This contribution is made to indicate the degree of alteration in dimensions of biologic specimens which can occur when they are mounted in fluid under a loose coverslip and the fluid is allowed to evaporate. I have frequently noticed that there is some increase; but had no idea that it amounted to as much, expressed in percentages, as is brought out by this test. Photographs were made of a ripe ovum of *Sarcoptes scabiei* at short time intervals as the fluid (water) evaporated, and all dimensions in the several photographs laid off on a scale with a compass. The findings are indicated in the table below. The photographs were taken at about four minute intervals under the heat of an Edinger apparatus.

	Length	Width
Photograph 1	0.190 mm.	0.130 mm.
Photograph 2	0.200 mm., 5.3% increase	0.138 mm., 6.2% increase
Photograph 3	0.208 mm., 9.5% increase	0.145 mm., 11.5% increase
Photograph 4	0.214 mm., 12.6% increase	0.150 mm., 15.4% increase

This means that such a difference in length as 12 per cent. and of width of over 15 per cent. is not to be overlooked when measuring biologic specimens either for comparison with known species or in describing new ones, and that the capillary force which is constantly being exerted more and more on a specimen should be remembered. The factor of pressure will of course vary in importance with different classes of material, depending on the shape, size and consistency of the object.

The dimensions recorded for any specimen should in all cases be as close as possible to those of the specimen as it exists in nature, i. e., as it lies in a stratum of fluid with something to protect it from the pressure of the coverglass. This can be accomplished coarsely by placing a minute drop of wax under the corners of the coverglass and warming over the flame as the coverslip is let down into position, or by ringing with vaselin. Finer adjustment of the thickness of the fluid stratum may be later accomplished under the microscope by hot wires applied to the wax pillars. In his work on protozoa, Doflein recommends such protection against pressure.

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* From the Laboratory of Dermatological Research, University of Pennsylvania.

NOTES

In the splendid volumes dedicated to Sir William Osler in honor of his seventieth birthday are some contributions of especial interest to parasitologists. Among these may be noted the papers on malaria control by Bass, on preliminary streptothricosis by Bridge, on spirochaetal jaundice by Gwyn, on leukocytes and protozoa by Goodrich, and on the significance of Rickettsia by Strong.

Professor Frank G. Haughwout, who for the past five years has been Professor of Protozoölogy and Chief of the Department of Parasitology of the College of Medicine and Surgery, and in the Graduate School of Tropical Medicine and Public Health, University of the Philippines, has transferred to the Bureau of Science, Manila. In the latter institution Professor Haughwout will be protozoölogist and will have supervision over the work in parasitology which it is planned to conduct on an extensive scale. Professor Haughwout will retain the position of professorial lecturer in protozoölogy in the University.

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NOTES AND EXPERIMENTS ON *SARCOCYSTIS TENELLA* RAILLIET

III. IS *SARCOCYSTIS TENELLA* AN ABERRANT FORM OF ONE OF THE CNIDOSPORIDIA OF INSECTS? *

John W. Scott

Several years ago in the first of this series of papers the writer published some results of certain experiments which seemed to favor Darling's insect theory in regard to infection of herbivorous animals with sarcosporidia. More recently it was shown that under Wyoming conditions *Sarcocystitis tenella* is subject to seasonal infection. Notwithstanding this fact the evidence adduced in this second paper was neutral so far as the insect theory was concerned. In the present paper a report will be made of a series of experiments which were intended to show by direct means that insects were responsible for infection with *Sarcocystis tenella*. There will follow a brief discussion of the significance of these experiments. It may be stated here that the results of the experiments were negative, or rather that infection took place independent of all conditions involving insects as the original hosts of the parasite.

The outcome of certain experiments described in the first of these papers led to a favorable opinion of Darling's theory that sarcosporidia of herbivorous animals are aberrant forms of Cnidosporidia that are normally parasitic in the intestines of insects. Accordingly, a series of experiments was arranged in 1915 with the following general plan. It was thought that if the infection was due to a parasite harbored in the intestine of native insects transmission to sheep could possibly take place by two or three methods. A lamb might become infected by eating insects, by eating grass or flowers contaminated with the excreta of insects, or possibly by drinking water in which the parasites had been set free through the death of insects. If the hypothesis proved correct the second method was probably the natural method of transmission, for eating an insect would be rare and accidental, and it would seem the accidental character of the third method would not be likely to account for the almost universal infection of our range

* Experiments by aid of the Adams Fund, Laboratory of Zoology and Parasitology, University of Wyoming.

sheep. The first method, moreover, would be in principle essentially the same as the second. Consequently, all lambs with their ewes, were kept in a dry lot until they were separated into three groups on June 25. Group I, consisting of ten lambs, was allowed to graze daily from June 26 to September 18 in a pasture (1) containing a permanent pond and considerable swampy ground. Previous work had shown that a large percentage of lambs allowed to run in this pasture became infected. Group II, consisting of eight lambs, were allowed to graze in a dry pasture from June 26 to September 18. This pasture contained no water and no swampy ground. Both groups, before and after dates mentioned, were kept in a dry, bare lot, fed baled native hay, a little grain, and given city water derived from deep springs. Group II received city water all summer. Group III, consisting of nineteen lambs, was kept in a dry bare lot all summer, fed baled native hay, given some dry grain, and furnished city water to drink. The details of their further treatment are shown in Table 1. In the first column is given the number of the lamb used in each experiment. In the second column is noted briefly the feeding or other special treatment that each received; tho not given in the table, some of these lambs received an additional treatment in reference to another experiment that dealt with tapeworm infection. This second procedure was unfortunate, as results showed, and tended to involve the problem, but it was thought that if *S. tenella* was a parasite due to insects, it is logical to conclude that the secondary treatment given could have no influence on infection. Accordingly, we shall give a brief explanation and discussion of the results first in relation to the insect theory and then in relation to the secondary treatment, the nature of which is mentioned below.

From the table it will be observed that insects were fed to lambs 203, 204, 205, 214, 216, 217, 221, 256, 257 and 258; that lambs 211 and 219 drank from aquaria contaminated with insects; that lamb 209 was fed flowers from the pasture where the lambs of Group I were allowed to graze; that lamb 210 was fed grass from the same pasture. Perhaps lambs 222 and 224 should be included in this list, since pen 8, from which they were fed grass, is located in pasture 1. Search was made for insects in this pasture twice each week during the summer. A short, cold, wet season contributed to the scarcity of most groups of insects, and only certain flies and mosquitoes were abundant. So far as the insect theory is concerned, lambs 213, 225 and 227 may be regarded as control. The fourth column of the table gives the number of feedings, twenty-two being the maximum for the summer. Finally, in column five the absence of infection, or if present the apparent degree or amount, is designated. The relative amount of

TABLE 1.—OUTLINE OF EXPERIMENTS IN 1915

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
203	Flies	862	22	Mild
204	Moths, butterflies	85	16	Very light
205	Moth larvae	2	2	Light
258	Bees, wasps	32	8	Very light
256	Mosquitoes	1,098	22	None
257	Ants	22	9	None
	Beetlees	19	10	
	Dragonflies	28	9	
	Grasshoppers	9	7	
	Hemiptera	59	9	
	Spiders	33	14	
209	Flowers, pasture I	....	22	Mild
210	Grass, pasture I	....	22	Mild
211	Water, aquarium V, containing killed insects	....	9	None
214	Nosema apis (in 5 dead bees)	Very large	1	None
216	Nosema apis (in 5 dead bees)	Very large	1	Very light
217	Nosema apis (in 5 dead bees)	Very large	1	Mild
221	Nosema apis (in 3 dead bees)	Very large	1	Heavy
219	Water, aquarium IV (Nosema opis in 4 bees)	....	9	None
213	Water, aquarium II (heart muscle containing sarcocysts)	....	11	None
222	Grass, pen 8; feces and proglottids	....	10	Very light
224	Same as 222	....	9	None
225	Grass and 6 insects; from small screen cage; sod contaminated with feces and proglottids	....	3	None
227	None	....	..	None

infection expressed where infection is present should be regarded as of comparatively little value, as this is not based upon numerical data.

In order to account for the wide distribution of sarcosporidia in herbivorous animals, Darling (1915) proposed the hypothesis that herbivorous sarcosporidia were merely aberrant cnidosporidia of insects, and suggested that the habit of depositing excreta on flowers and grass by certain insects, such as bees, moths, etc., would probably be found a sufficient explanation to account for infection. If this were true, feeding the insects ought also to produce the infection. This was the basis of the series of experiments in 1915. A modification of this plan is shown in the case of lambs 211 and 219, where the lambs were made to drink from aquaria into which dead insects were thrown, and in the case of lambs 209 and 210, which were fed very considerable quantities of flowers and grass that had been exposed to insects in pasture 1. This last method would conform to normal infection according to Darling's hypothesis. A close study of the results gave no definite indication that insects either were or were not the cause of infection. For infection apparently resulted from feeding 862 flies, from feeding 85 moths and butterflies, from feeding 2 moth larvae, from feeding 32 bees and wasps, from feeding to each of three lambs 3 to 5 bees that had died with the Isle of Wight disease which is due to the Cnidosporidian, *Nosema apis,* from feeding flowers, from feeding grass, but it did not occur as the result of feeding 1,098 mosquitoes, or as the result of feeding various other insects and arachnids, including ants, beetles, bugs, dragon flies, grasshoppers and ground spiders.

infection also failed in one lamb after feeding 5 bees that had died from infection with *Nosema apis*. Both lambs exposed to aquaria containing various killed insects, including bees infected with *N. apis*, remained uninfected. If infection was due to insects it was derived from three widely different orders namely, Diptera, Lepidoptera and Hymenoptera, and it occurred as the result of eating insects or as the result of eating flowers and grass exposed to insects. One might suppose that the infection was derived from spores deposited on the hay by insects before it was baled. But considering the delicate character of the spores, this was not thought probable. Or, one might suppose the infection due to excreta of certain insects (some flies) that visit dry lots as well as pastures. One thing appeared certain: the *infection with S. tenella could not be due to N. apis.* For infection occurred independent of this parasite (lambs 203, 204, 205), and in one case it did not occur in a lamb when the parasite was known to be present.

Any other theory to account for infection, based on the special treatment received by the lambs, appeared equally unsatisfactory. Of sixteen lambs fed grass or water contaminated with feces, by the secondary treatment mentioned, eight were infected and eight not infected. Lambs 203 and 204 which had received tapeworm proglottids were both infected; the tapeworms appeared to be clean, but of course they might carry some fecal contamination. But if the infection occurred thru contamination with feces, one may well ask why were not all the lambs infected? For all lambs of Group III were exposed to the feces of the ewes running with them in the same lot, and we know that nearly 100 per cent. of our range ewes are infected with *S. tenella*. On the whole, the experiments were deemed inconclusive, since we had not been able to control the infection.

Mention should be made of the results in lambs of Groups I and II. In Group I, including the lambs which were allowed to graze in a pasture containing a pond and some swampy ground from June 26 to September 18, six out of ten lambs were found with sarcocysts. In Group II, grazed in like manner in a dry pasture, sarcocysts were found in only three out of eight lambs. These results indicated that moist or swampy pasture conditions favored infection. The significance of these results became more apparent in later work. Not much emphasis should be placed on these results, for stained sections were not prepared, and subsequent work has shown that some parasites (and probably some infections) will be overlooked unless this is done.

The unsatisfactory outcome of the year 1915 led to a new series of experiments in 1916. The failure to gain control over infection emphasized the importance of carrying out the details of the experiments in a very careful, rigid manner. Again the lambs with their

accompanying ewes were divided into three groups, but the groups received somewhat different treatment. Group I, consisting of six lambs and several ewes, was allowed to graze during the summer in pasture 1. The pasture, however, had been altered in the following way: the pond previously mentioned together with a grassy slope above it was fenced off, and this fenced-off portion will hereafter be called lot III. The lambs of Group I therefore had daily access during the summer to a pasture containing considerable wet and swampy ground. Group II consisted of 22 lambs which were kept in a dry, bare lot from the time they were born until they were killed; they were given dry grain feed, watered in troughs with city water, and up until about September 15 were fed native hay kept over from the previous season; after this date these lambs were fed hay cut during the summer of 1916, since the old hay could no longer be obtained. The ewes to which these lambs belonged were kept with them in the dry lot. The individual treatment of this group of lambs is shown in Table 2, and will be mentioned later; Group III, consisting of six lambs, numbered consecutively 261 to 266, were dept in a dry, bare lot and pastured twice per week in lot III mentioned above. This lot was about 70 feet wide by 100 feet long; the pond and its borders covered most of the lower third on one side; the remainder was a dry grassy slope from 2 to 7 feet above the pond. No ewes or lambs had been on this lot since the previous season, and no ewes were allowed in lot III during 1916. So the lambs of Group III had contact with ewes only while they were in the dry lot.

TABLE 2.—INFECTION IN 1916

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
72	Grass from pasture I	....	15	Very light
73	Flowers from pasture I	....	15	Rather light
74	Flies	1,183	15	Light
75	Mosquitoes	137	7	Medium
76	Moths	65	13	None
77	Butterflies	10	5	Very light
78	Lepidoptera larvae	68	10	None
79	Bees	7	4	Light
80	Wasps	27	8	Very light
82	Ants	12	3	Light
	Beetles	482	12	
	Bugs	44	8	
	Dragonflies	37	13	
	Grasshoppers	54	14	
	Spiders	67	10	
81	Control	....	....	Very light
86	Control	....	....	None
	Control	....	....	Very light
85	Control	....	....	Very light
268	Control	....	....	Light
269	Control	....	....	Light
270	Control	....	....	None
271	Control	....	....	Light
272	Control	....	....	Light
267	Water from pond in lot III	....	12	Very light
273	Grass in pen, lot III	....	13	Medium
275	Grass in pen, lot III	....	13	Very light

Only lambs of group II are shown. See text for other groups.

An inspection of Table 2 shows a plan quite similar to that of 1915. Lamb 72 received grass and lamb 73 received flowers on fifteen different days between July 18 and September 23. At each feeding, which was done individually and by hand, the parts of flowering plants obtainable in pasture 1 were selected with the idea that they had been contaminated with the excreta of insects. With a similar idea numerous tufts of grass from different parts of the pasture were collected for each feeding of lamb 72. During the season one lamb was fed 1,183 flies; another 137 mosquitoes; another 65 moths; another 10 butterflies; another 68 lepidoptera larvae; another 7 bees; another 27 wasps, and still another was fed a miscellaneous group of insects including 12 ants, 482 beetles, 44 bugs, 37 dragonflies, 54 grasshoppers and 38 spiders. Nine lambs, numbers 81, 84, 85, 86, 268, 269, 270, 271, 272, received no special treatment, and were kept as a control. On twelve days lamb 267 was given water from the pond in lot III. On thirteen different days between July 17 and September 11 two lambs, numbered 273 and 275, were grazed in a small pen located in lot III. This pen had formerly been used as a feed pen for small pigs, but had not been used for several years; it was 10 by 12 feet in size, overgrown with grass, and no ewes or lambs had been inside of it for at least two years; it was located on the dry grassy slope of lot III some distance above the pond.

The lambs were all killed during the following winter at various times between November 3, 1916, and March 16, 1917. Material was preserved, sectioned, stained and carefully examined for parasites. The last column of Table 2 gives the general results. Group I, consisting of six lambs that grazed all summer in pasture 1, showed 100 per cent. infection. Group III likewise showed 100 per cent. infection; these lambs were grazed on fifteen different days, between July 17 and September 11, in lot III which surrounded the small pond. Of the two lambs grazed in the small pen in lot III, both were infected. So also were lambs 72, fed grass, and 73, fed flowers. Lamb 267, watered twelve times from the pond in lot III, showed a light infection. Of the nine lambs composing the control seven were infected, and the parasite was found in six out of eight of the lambs which were fed insects. Infection therefore took place independent of the control, and feeding insects did not have any discernible effect either in the number of lambs infected or in increasing the heaviness or degree of infection. A comparison with the lambs fed insects the previous year furnishes some further evidence that insects were not related to infection. For example, lamb 205 (1915) had a light infection after feeding only two moth larvae, while no infection was found in lamb 78 (1916), altho it had received 68 lepidopterous larvae, mostly those of moths. Again, no infection resulted after the feeding of 1,098 mos-

quitoes to lamb 256 in 1915, while feeding 137 of the same insects was followed by a medium infection in lamb 75 in 1916. The feeding of a miscellaneous group of insects and arachnids apparently produced no infection in lamb 257, and the same treatment given to another lamb the next year was followed apparently by a light infection.

From these results it was evident that infection occurred independent of the experiments that were planned to show the direct connection of insects with *S. tenella*. So if insects were responsible for infection, the parasite must be limited to such insects as were common to all of the general conditions present. Now the insects commonly present in the dry lots were limited to certain species of flies, principally houseflies and stable flies, and these were also present in the pastures and lots used. But such evidence as we had was rather opposed to the idea that these insects were responsible for the infection. For 862 flies were fed to lamb 203, and 1,183 flies were fed to lamb 74, and neither of these lambs showed more than a moderate degree of infection; while the number of flies fed included several different species, houseflies and stableflies formed a considerable part of the total number. It was seen, therefore, that we had found no evidence favoring the insect hypothesis. But since the character of the evidence was largely negative there still remained the possibility that the theory might be true.

Accordingly, in the next series of experiments it was planned to arrange the details in such a way that if infection occurred it would exclude the possibility of infection by insects. At the same time some insect and flower feeding experiments were continued. Table 3 shows the general details of these experiments. Mosquitoes were fed twice weekly to lamb 282. Flies were fed to lamb 286; bees to lamb 288; flowers to lamb 289. Flowers were also fed to lamb 287, but in this case the flowers were first heated, with the idea of killing any organisms that might be present, and then exposed to insects for several hours in a closed jar; several species of insects were included, but the common flies caught about the lots formed a large part of the number. By this means the grass was well contaminated with the feces of insects. Lamb 286 unfortunately died from an undetermined cause on July 28, after only four feedings of flies; no sarcocysts were found. The other lambs mentioned were fed twice a week as long as insects or flowers were available. Three other lambs a few days after they were born and before insects appeared, were placed in a screen cage where they were kept until long after all insects had disappeared. Of these lambs, No. 300 was fed flies twice a week, beginning July 17 and ending September 29; altogether, it was fed 1,797 flies which for the most part were caught around the dry lots. Lambs 285 and 287 were kept in the screened cage as controls and with no

other treatment. Two ewes, 87, the mother of lamb 287, and 176, the mother of lambs 285 and 300, were also kept in the screened cage. During the summer a small number of houseflies gained access to the inside of the screen cage, but as these were killed as soon as discovered it is believed they did not have any effect on the experiment.

TABLE 3.—Treatment and Infection in 1917

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
282	Mosquitoes	1,809	16	Medium
286	Flies	82	4	None, died July 28
288	Bees	384	16	Light
289	Flowers	....	17	Light
297	Flowers sterilized; exposed to insects	....	17	Light
300	Kept in screen cage; fed flies	1,787	20	Light
285	Kept in screen cage; control	....	....	Light
287	Kept in screen cage; control	....	....	Heavy

Lambs 282, 286, 288, 289 and 297 were kept in a dry lot all summer.

When the lambs were killed it was found that all were infected with *S. tenella* except lamb 286 which died early. Here again it was evident that we had been unable to control infection. The two control lambs, 285 and 287, were both infected, and in these cases infection had clearly occurred without the aid of insects. In fact, lamb 287 had a heavier infection than any other lamb of the season. Again, as in previous seasons, the feeding of insects produced no noticeable effect on infection. Assuming the insect theory to be correct and that *S. tenella* is an aberrant form, one would expect that if any lambs in the screen cage were infected this would be true only of lamb 300; but such was not the case. On this theory one would expect lamb 297 to show a heavy instead of a light infection, for the sterilized plants upon a number of occasions were very generously contaminated with the feces of insects.

From the results of these three series of experiments it was believed that *Sarcocystis tenella* could not be considered an aberrant form of the Cnidosporidia, for infection apparently occurred without the presence of insects. Consequently, Darling's hypothesis on which we had been working was probably no longer tenable. However, considering the small number of lambs raised in the screened cage, it was thought desirable to obtain additional proof. So in the following year four lambs and their ewes were put in a screened cage and kept there until the lambs were killed.

Lamb 1 was born March 14 and put in the cage the following day; lamb 2 was born May 1 and put in the cage May 7; lamb 3 was born May 4 and put in the cage ten days later on May 14; lamb 6 was born May 10 and put in the cage next day. When killed, lambs 1 and 3 showed a medium degree of infection, while lambs 2 and 6 had fewer sarcocysts present.

These lambs were all put in the cage before insects appeared. From time to time one or two houseflies found a way into this cage, but as a daily search was made for such accidental visitors and they were killed as soon as found, it was believed in view of previous results that they did not have any influence on infection with *S. tenella.* On a few occasions, and for a short time only in the early summer, small gnats were observed in the cage; these had been small enough to pass thru the fine meshes of the screen. The ephemeral character of these insects would indicate that they did not harbor Cnidosporidia that would develop into *S. tenella* if transferred to the sheep. But in spite of all precautions to prevent a possible infection by insects, all four lambs were found infected when killed. Of the seven lambs that had been raised in screened cages all, or 100 per cent., were infected; this had not been true of lambs raised in dry lots outside. The percentage of infection had been increased by raising lambs in a screened cage, even tho all possible precautions were taken to protect these lambs from insects. Except for the slight possibility of infection by means of the accidental insects mentioned above, there seemed to be no longer any ground for holding to Darling's theory. There remained only one more experiment to make the proof complete. That was to raise infected lambs entirely independent of insects.

Lamb 97, born January 23, and lamb 98, born January 24, were placed in a screened cage on April 5. There were no flies in the cage at any time before the lambs were killed on July 1, and except for some small gnats and a few small mosquitoes during the latter half of June, no insects were present. As a rule, on the Laramie Plains practically no insects are present before the first of June, and they are seldom common until after the middle of June. Since it is well known that *S. tenella* requires at least four weeks to appear in the muscles after infection, it is clear that such insects as were present could have had absolutely no influence on infection. Nevertheless, lamb 98 was found lightly infected, and one of the sarcocysts was not less than two weeks old. Hence, one is forced to the conclusion that *Sarcocystis tenella* is not an aberrant form of the Cnido-sporidia of insects.

While one can now safely reject the idea that *S. tenella* is an aberrant Cnidosporidian there are still left other important questions to be answered. Does the life history of *S. tenella* require an intermediate host? By what means do lambs become infected? What is the probable life history of this parasite? These and other questions will be discussed in the next paper, after another series of experiments has been described. It is, however, desirable to mention the relation of the results found in this paper to seasonal infection. It is now apparent that the seasonal infection described in the second of this series of papers does not depend upon insects, and the conditions in some of

the experiments strongly favor the idea that no intermediate host is necessary. If this is true the hypothesis that there is an infective stage in the feces of the sheep, similar to that demonstrated for *S. muris* by Nègre and Crawley, acquires added importance, and the delicate nature of the spores as noted by Fantham and Porter, coupled with adverse climatic conditions, is probably sufficient to account for seasonal infection.

It may be mentioned that lambs 97 and 98 furnished a little additional evidence on seasonal infection. Altho more than 22 weeks old when killed July 1, no sacrocysts were found in lamb 97, and the size of the sarcocysts in lamb 98 indicated that infection occurred not earlier than seven or eight weeks previous to this date. While warm periods lasting a week or two may come as early as May or the latter part of April, freezing at night and snows are common up to the first of June. Considering that these lambs were kept under conditions that always had resulted in infection in 100 per cent. of the lambs used, and remembering that infection is rather common in late spring lambs after they are 10 to 14 weeks old, it appears that the limitation of infection in lambs 97 and 98 was due to seasonal influences.

I am indebted to my assistant, Mr. E. C. O'Roke, for a considerable part of the routine work involved in the experiments mentioned in this paper. I am indebted to the Bureau of Entomology, Department of Agriculture, Washington, D. C., for the bees infected with *Nosema apis*.

SUMMARY

1. *Sarcocystis tenella* is apparently not an aberrant form of one of the Cnidosporidia of insects, for lambs become infected with this parasite without insects being present. Darling's hypothesis is therefore probably untenable.

2. It has been found that lambs are more certain of becoming infected, and that the number of parasites per unit of muscle is greater if they are kept closely confined in a screened cage than if they are allowed to run free in an open dry lot.

3. A second host other than the sheep does not seem necessary for the development of *S. tenella*, and this being true, a sexual stage of this parasite will no doubt be found in the intestine of the sheep. The method of transmission and life history will be taken up in the next paper.

NOTES ON THE LIFE CYCLE OF TWO SPECIES OF ACANTHOCEPHALA FROM FRESHWATER FISHES *

H. J. Van Cleave

The complete life cycle is not known for a single typically North American species of Acanthocephala. Species which have served as the basis of work by investigators on other continents have invariably revealed a complicated cycle of development involving at least one other host in addition to the definitive host which shelters the mature worms. Frequently intermediate hosts have been found intercalated between the primary and the definitive hosts. In most species of Acanthocephala there is not an absolutely fixed specificity of hosts, either primary or definitive. Consequently it is probable that species for which the life cycle has been determined in another country may have acquired entirely different species of hosts on this continent. For example, *Gigantorhynchus hirudinaceus* (Pallas), the common acanthocephalon of the hog, has an extremely broad geographical distribution, but it must utilize various primary hosts within the limits of its range for the geographical distribution of the insect larvae which serve as primary hosts in any given locality does not coincide with the geographical distribution of the parasite. Thus Stiles (1892) found that the species of insect larvae which act as primary hosts for this parasite on this continent are entirely different from the ones that are utilized in its European habitat. For similar reasons one could not be justified in assuming that representatives of the species *Echinorhynchus gadi* Müller from fishes of our Atlantic coast undergo a development identical in detail with representatives of the same species from the European continent.

The works of Linton are the only published records which include information concerning larvae of Acanthocephala peculiar to North America. His references are based chiefly upon incidental observations of larvae found in marine fishes. His frequent mention of *Echinorhynchus incrassatus* from the viscera of various species of marine fishes (1891, 1901, 1905) unquestionably refers to the larvae of an undetermined species of Corynosoma as evidenced by his own drawings. He also recorded the occurrence of *"Echinorhynchus proteus"* (1901 : 481) in the mesentery of a flounder. This last may without doubt be referred to the genus Pomphorhynchus, though the specific identity with the European *P. laevis* (= *"proteus"*) is to be

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*Contributions from the Zoological Laboratory of the University of Illinois, No. 150.

doubted. Thus two of the larval parasites mentioned in Linton's works stand without any definite relationships to species known to occur as adults in the North American fauna. In addition to the above mentioned records, Linton described (1889) a new species of Acanthocephala, *Echinogaster sagittifer,* from larvae which were encysted in the viscera of marine fishes. At a later date he definitely associated these larval forms with adults which were subsequently discovered. The larvae from which the species was described were in an advanced stage of development, ready to infect the definitive host. Consequently even in this instance of a marine species of Acanthocephala peculiar to the North American continent little is known of the details of its life cycle. Yet this represents as full an account as is to be found in literature, while for the purely freshwater species nothing has been recorded. In no instance has a complete life cycle been outlined and verified by data from experimental infestation of either primary or definitive host.

In the course of extensive laboratory and field investigation upon Acanthocephala, the writer has come into possession of facts relating to the life cycle of two species of these parasite from freshwater fishes. In view of the fact that no data concerning such forms is available in the literature, it seems advisable to publish the results of this investigation in the hope that other investigators may be induced to cooperate in bringing together data bearing upon the development of other species of these parasites. The present paper embodies information relating to certain phases in the life cycle of two species belonging to the genus Echinorhynchus, both of which are apparently peculiar to freshwater fishes of North America.

SOURCES OF DATA

Investigators in specialized fields frequently fail to realize the service they might render to other investigators by making known to them the incidental or accidental information that they may acquire in the pursuit of their special problems. The materials which formed the basis for the present study have in great measure come to the writer through the cooperation of friends who secured the data while carrying on investigations directed along fundamentally different lines. I am under especial obligation to Drs. A. S. Pearse, A. R. Cooper, G. R. La Rue and Director A. F. Shira for specimens and for data concerning the occurrence of larval Acanthocephala. The U. S. Bureau of Fisheries during the summer of 1919 extended to the writer the opportunity of carrying on investigations concerning the life histories of Acanthocephala infesting fishes, but that work has not progressed to a stage where positive data are available from the experiments that were undertaken.

THE LARVA OF *ECHINORHYNCHUS COREGONI* LINKINS

In the course of investigation upon parasites of fishes, Dr. A. R. Cooper encountered heavy infestations of Acanthocephala in the digestive tract of whitefish taken from the Great Lakes region, especially from the Canadian shore of Lake Ontario. The same individuals which bore heavy infestations of these parasites contained numerous amphipods (*Pontoporea hoyi*) in their stomachs. An examination of some of the amphipods revealed the presence of larval Acanthocephala, which, according to unpublished observations by Dr. Cooper, were located in the body cavity. I have examined specimens, both from the body cavities of these amphipods and from the intestine and ceca of the fishes, and have identified them as belonging to the species *Echinorhynchus coregoni* Linkins. The larval forms taken from the bodies of the amphipods were in a late stage of development. In fact, the size and plan of organization of these larvae did not in any manner differ from the juvenile representatives of the same species taken from the intestine and ceca of the definitive host. All of the larvae which I examined had the proboscis fully retracted within the body, so that serial sections were necessary for accurate determination.

Early stages in the development of this species, from the time the embryo leaves the body cavity of the gravid female to the infecting stage, remain unknown. It seems probable that the entire larval existence may be passed in the body of an amphipod which serves as primary host, and that the larva is introduced into its definitive host when some fish, suitable as a definitive host, devours the infected amphipod.

THE LIFE CYCLE OF *ECHINORHYNCHUS THECATUS* LINTON

A number of years ago Director A. F. Shira of the Fairport, Iowa, Biological Station, encountered larval Acanthocephala in amphipods (*Hyalella knickerbockeri*) which he was rearing as food for young small-mouthed black bass at the Homer (Minnesota) station of the U. S. Bureau of Fisheries. The appearance of these larvae in the amphipods was coincident with an outbreak of an epidemic of acanthocephalan infestation among the young bass. Specimens of the parasites, both from the alimentary canal of the fish and from the bodies of the amphipods, were preserved. Through the courtesy of Director Shira, I have been permitted to examine a few of these specimens and have determined that the mature worms from the bass and the larvae from the amphipods both belong to the species *Echinorhynchus thecatus* Linton. The demonstration, in this instance, of the occurrence of larvae of *E. thecatus* in amphipods and the general infestation of the young bass which were being fed these same amphipods under controlled conditions lends strong support in advocacy of the con-

tention that *E. thecatus* may undergo its complete cycle of larval development within the body of an amphipod and reaches maturity when a suitable definitive host devours the amphipod which shelters the infecting stage of the larval parasite.

Data from other sources furnish incontestable evidence that one or more intermediate hosts may be intercalated between the primary and the definitive hosts of *Echinorhynchus thecatus*. Larvae which unmistakably belong to this species have been encountered frequently encysted in the viscera of various fresh water fishes. At the present time no proof of the method by which they come into this host is available. However, it seems probable that they enter in the same manner as found in other species of these parasites. If an amphipod bearing young larvae of *E. thecatus* is eaten by a fish the young larvae would be liberated in the digestive tract of the fish, but being imperfectly developed would not be able to maintain themselves as intestinal parasites of the new host. Such early larvae probably penetrate the wall of the digestive tract of their new host and become encysted in the mesenteries, in the peritoneum, or in the organs lying in the general body cavity. Here they continue development until they reach a stage identical with the fully formed infecting larvae that occurs in the amphipod.

Evidence in support of the above outlined stage in the life cycle of *E. thecatus* has been derived from the study of material collected by Dr. A. S. Pearse who encountered numerous cysts of various size in the peritoneum of the yellow perch. Pieces of the peritoneum containing these cysts were stained and mounted for study. Many of the cysts included numerous hooks (Fig. 2) which were directly recognizable as the proboscis hooks of *E. thecatus,* while others, more fully developed, contained complete larvae (Fig. 3) in the infecting stage. The writer expects at some later date to examine the living cysts and to supplement the present study by an examination of serial sections of these encysted larvae.

The smallest cyst in the material examined which has been definitely recognized as containing a larva of *E. thecatus* is 0.18 mm. in diameter and almost spherical in form (Fig. 2). A definite fibrous wall surrounded this cyst, and others of a similar structure. In the cyst shown in this figure no definite arrangement of the hooks could be observed, though in many of the more advanced stages the inverted proboscis could be definitely recognized in the toto-mounts of the cysts.

In many of the largest specimens, which frequently attained a length of approximately 2 mm., the proboscis of the larva was fully extended as shown in Figure 3. Individuals such as the one shown in this figure represent the end of the developmental processes of the larva, for they have attained the full size and the same organization

of the body as found in the juvenile specimens of the same species removed from the lumen of the intestine of a definitive host (see Fig. 4).

It is significant that fully formed larvae of the infesting stage are found in Hyalella, while much smaller, less fully developed specimens have been observed in a vertebrate which serves as an intermediate host. This would indicate that the intermediate host in this instance serves not only as a repository for larvae which accidentally enter its body, but also furnishes suitable conditions for completion of the development of the immature larvae which has not gone far on its course when its sheltering primary host is eaten by the intermediate host.

Attainment of the adult body form from a juvenile, such as shown in Figure 4, involves practically no change in the proboscis. Simple increase in size of the body proper and development of the reproductive organs from the rudiments present in the larva constitute the chief developmental processes that proceed after the larva has entered the digestive tract of its definitive host. Since many of the characters utilized in the classification of the Acanthocephala are associated with the proboscis, the early development of this organ renders an identification of larvae possible even though the general body form may be grotesquely different from that of the adult.

The writer (1919) has previously discussed the occurrence of larvae of *E. thecatus* in the viscera of fishes from Douglas Lake, Michigan. Larvae of this species from various organs of fishes examined by Dr. G. R. La Rue are identical in structure with the juvenile forms of the same species which were taken from the digestive tract of definitive hosts from the same locality.

Stages in the development of *E. thecatus* earlier than the one shown in Figure 2 have not been discovered. The form of the larva from the time it leaves the body of the gravid female parasite (Fig. 1) until it reaches this comparatively late stage in its cycle remains unknown.

SUMMARY

1. Some phases in the life cycle of *Echinorhynchus coregoni* Linkins and of *E. thecatus* Linton have been made available, chiefly through the cooperation of other investigators.

2. Larvae of *E. coregoni* have been found in *Pontoporea hoyi*.

3. Pontoporea containing these larvae were taken from the stomach of whitefish which carried an infestation of this some parasite in the digestive tract.

4. Larvae of *E. thecatus* have been found in *Hyalella knickerbockeri*. The young bass fed on these amphipods acquired a general infestation of *E. thecatus*.

5. Various fishes harbor encysted larvae of *E. thecatus* in their viscera and in the peritoneum. An intermediate host does not seem essential for the completion of the life cycle of this parasite.

6. In these two species the transformation from fully formed larva to the adult involves conspicuous changes in the body proper, but practically none in the proboscis.

Literature Cited

Linton, E. 1889.—Notes on Entozoa of Marine Fishes of New England, with descriptions of several new species. Rep. U. S. Fish Com. for 1886, 14: 453-511.

1891.—Notes on the Entozoa of Marine Fishes with Descriptions of New Species, Pt. III. Rep. U. S. Fish Com. for 1888, 16: 523-542.

1901.—Parasites of Fishes of the Woods Hole Region. Bull. U. S. Fish Com., 19: 405-492.

1905.—Parasites of Fishes of Beaufort, North Carolina. Bull. Bur. Fish., 24: 323-428.

Stiles, C. W. 1892.—Notes on Parasites. On the American intermediate host of *Echinorhynchus gigas*. Zool. Anz., 15: 52-54.

Van Cleave, H. J. 1919.—Acanthocephala from the Illinois River, with descriptions of species and a synopsis of the Family Neoechinorhynchidae. Bull. Ill. Nat. Hist. Survey, 13: 225-257.

1919a.—Acanthocephala from Fishes of Douglas Lake, Michigan. Occas. Pap. Museum Zool. Univ. Mich., 72: 1-12.

Explanation of Plate XIV

Stages in the life-cycle of *E. thecatus*

All figures were drawn with the aid of a camera-lucida from permanent, stained toto-mounts in balsam. Scales accompanying Figures 1 and 2 have the value of 0.05 mm., the others have the value of 0.1 mm.

Fig. 1.—Hard shelled embryo from body cavity of gravid female. Embryos are discharged in about this stage. Cleavage has progressed to a considerable extent.

Fig. 2.—Young larval cyst from peritoneum of yellow perch.

Fig. 3.—Late larval stage ready to infect definitive host. Larva embedded in peritoneum of yellow perch.

Fig. 4.—Juvenile form removed from intestinal cavity of yellow perch. This and foregoing figure drawn to the same scale show identity of form in late larva and juvenile from definitive host.

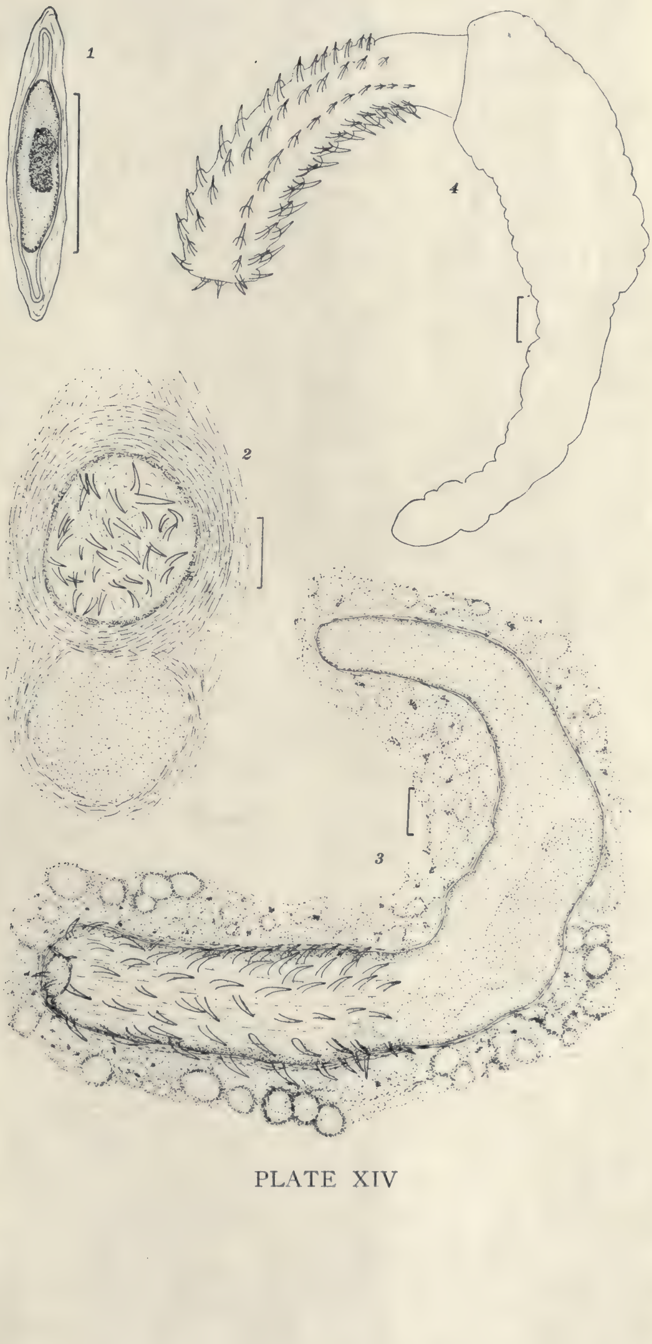


PLATE XIV

SUR LA SOURCE D'INFECTION DU CHIEN ET DU CHAT AVEC *L'ECHINOCHASMUS PERFOLIATUS* (V. RÀTZ) ET LA QUESTION D'INFECTION DE L'HOMME AVEC LES DISTOMES DE LA FAMILLE DES ECHINOSTOMIDÉS

NOTE PRÉLIMINAIRE

PAR J. CIUREA

A ma connaissance la source d'infection de l'homme et des animaux avec les Echinostomes n'est pas encore expérimentalement déterminée; on croyait seulement que cette infection était produite par la consommation de mollusques infestés par des cercaires d'Echinostomes, ou par la boisson des eaux dans lesquelles se trouveraient ces cercaires. Jusqu'à présent on ignorait que les poissons et spécialement ceux d'eau douce pouvaient être considérés comme source d'infection avec ces Distomes.

Dans ce qui suit je veux faire connaitre la source d'infection avec *l'Echinochasmus perfoliatus* v. Ràtz, parasite de l'intestin du chien, du chat et du porc.

Au cours des années 1913-15 nous nourri des chiens et des chats avec différentes espèces de poissons du Danube, dans le but de déterminer lesquels de ces poissons servent comme hôtes intermédiaires aux larves des Opisthorchiidés; et à l'occasion de ces recherches, à l'autopsie des animaux d'experience, nous avons trouvé plusieurs fois dans leurs intestins grêles des exemplaires de *l'Echinochasmus perfoliatus*.

En ce qui concerne les animaux d'expérience (chiens et chats) nous tenons à mentionner qu'ils étaient de jeunes sujets, en grande partie élevés par nous, et qui avant l'expérience n'avaient jamais mangé de poissons, de mollusques ou bu d'eau dans laquelle l'on pouvait soupçonner la présence de mollusques. Pendant nos recherches, ces animaux ont été tenus dans des cages séparées. Comme nourriture ils recevaient des poissons crus, et quand nous n'en avions pas nous leur donnions du foie de bouf. Cette nourriture leur était distribuée par nous-même.

Les expériences qui font le sujet de notre travail sont les suivantes:

Première série d'expériences avec le *Scardinius erythrophthalmus* et la Brême (*Abramis brama*).

Trois petis chiens de la même nichée, dont l'un a été nourri depuis le 5 Avril jusqu'au 17 Juin (74 jours) avec *Scardinius erythrophthalmus*, un autre chien pendant la même période de temps fut nourri avec 5 grands exemplaires d'*Abramis brama* et le troisème chien nous servit comme contrôle.

Le résultat de l'autopsie des ces trois chiens qui eut lieu le 19 Août (137 jours après le commencement des expériences) fut le suivant: Dans le contenu intestinal du chien nourri avec le *Scardinius erythrophthalmus* nous avons trouvé 5 exemplaires d'*Echinochasmus perfoliatus;* dans celui qui fut nourri avec de la Brême seulement 1 exemplaire; et chez le chien contrôle on ne trouva aucun Trematode dans l'intestin. Le contenu intestinal fut toujours conservé dans 70% alcool et examiné avec la plus grande attention au microscope.

Deuxième série d'expériences. Des chiens et des chats nourris avec la Tanche (*Tinca tinca*), le Brochet (*Esox lucius*), le Carassin (*Carassius carassius*) et l'*Aspius aspius* (l'année 1913).

Deux petits chiens, l'un d'eux a été nourri depuis le 16 Août jusqu'au 3 Novembre avec de la Tanche (*Tinca tinca*). Il a mangé 71 exemplaires de cette espèce de poisson pendant 80 jours. Son frère reste comme controle. A l'autopsie des ces deux chiens sacrifiés le 4 Novembre, j'ai recueilli de l'intestin du chien qui a mangé de la Tanche 1488 exemplaires d'*Echinochasmus perfoliatus.* Chez l'autre chien contrôle on n'a trouvé aucun Distome.

Trois petites chattes soeurs dont l'une a consommé en l'espace de 66 jours (du 1^er^ Septembre au 6 Novembre) 44 Brochets (*Esox lucius*), une autre a ingéré en 60 jours (du 23 Septembre jusqu'au 23 Novembre) 88 Carassins (*Carassius carassius*) et la troisième a servi pour le contrôle.

Après les avoir sacrifiées nous avons recueilli 33 exemplaires d'*Echinochasmus perfoliatus* de la chatte qui fut nourrie du Brochet et 5 exemplaires de celle qui mangea du Carassin. A la chatte contrôle on ne trouva aucun Echinostome.

Deux petits chiens frères dont l'un a été nourri pendant deux mois (depuis le 23 Octobre jusqu'au 23 Décembre) avec 55 exemplaires d'*Aspius aspius,* l'autre servit de contrôle. A l'autopsie du chien nourri d'*Aspius aspius* on trouva dans le contenu intestinal 67 exemplaires d'*Echinochasmus perfoliatus,* chez le chien contrôle aucun exemplaire.

Troisième série d'expériences. Des chiens nourris avec l'Ide jesse (*Idus idus*), Carpe (*Cyprinus carpio*), Barbeau (*Barbus barbus*) et *Blicca björkna* l'année 1913-15.

Quatre chiens dont l'un a mangé depuis le 4 Novembre 1913 jusqu'au 31 Mars 1914 (5 mois) 14 exemplaires de l'Ide jesse (*Idus idus*). A l'autopsie de ce chien on trouva dans l'intestine 540 exemplaires d'*Echinochasmus perfoliatus.* Dans l'intestine du deuxième qui a ingéré pendant plus l'ume anné (27 Décembre 1913 jusqu'au I Février 1915) 65 Carpes et dans l'intestin du troisième chien qui fut nourri dans le même espace de temps avec 19 Barbeaux on n'a trouvé aucun exemplaire d'*Echinochasmus perfoliatus.* Enfin au quatrième chien qui mangea depuis le 17 Juin jusqu'au 23 Juillet (36 jours) 66 *Blicca björkna* on trouva dans l'intestin 3 exemplaires d'*Echinochasmus perfoliatus.* Chez le chien contrôle on n'a rien trouvé.

Le résultat des ces expériences est que les chiens et les chats ont été infestés par *Echinochasmus perfoliatus* v. Ràtz en consommant les espèces suivantes de poissons du Danube: *Scardinus erythrophthalmus,* la Brème (*Abramis brama*), la Tanche (*Tinca tinca*), le Brochet (*Esox lucius*), l'*Aspius aspius,* l'Ide jesse (*Idus idus*) et *Blicca björkna.*

De même il résulte que les hôtes intermédiaires de prédilection des larves d'*Echinochasmus perfoliatus* sont la Tanche et l'Ide jesse.

Je veux faire remarquer que les poissons voraces, Brochet et *Aspius aspius,* étant toujours mangés en entier par les animaux d'expériences, il est possible que les exemplaires d'*Echinochasmus per-*

foliatus trouvés chez ces animaux proviennent d'autres espèces de poissons qui se seraient éventuellement trouvées dans leurs estomacs.

Après avoir pris connaissance des poissons qui portent les larves d'*Echinochasmus perfoliatus,* j'ai commencé à faire des recherches pour trouver les larves même. J'ai fait l'examen surtout de la Tanche et de l'Ide jesse puisq'il est résulté de mes expériences que ces poissons sont les hôtes de prédilection pour les larves d'*Echinochasmus perfoliatus.*

Par de minutieuses recherches je suis parvenu à trouver de très petites larves d'Echinostomes enchistés dans les écailles et seulement dans le canal de la ligne latérale, un organe qui n'était pas jusqu' à présent connu comme siège des parasites animaux.

Pour le moment je ne peux donner qu'une description sommaire des caractères de ces larves, mes recherches n'étant pas encore complètement terminées. Les larves sont incapsulées dans des chistes ellipsoïdes à double parois, les dimensions d'un tel chiste sont 0.197 mm. sur 0.147 mm. La larve retirée du chiste, un peu contractée mesure 0.197 mm. en longueur et 0.088 mm. en largeur. La surface de son corps présente de très fines épines. La ventouse orale placée à l'extremité antérieure du corps est un peu plus petite que celle ventrale qui se trouve près du milieu du corps. Le disquè adoral est réniforme et porte une série de 27 bâtonnets, petits, disposés sur un seul rang ininterrompu sur la face dorsale.

Maintenant vient la question si cette larve d'Echinostome est celle d'*Echinochasmus perfoliatus.* Ce qui s'oppose à cette identification est premièrement le nombre plus grand de bâtonnets du disque adoral de la larve (27) que celui des exemplaires adultes d'Echinochasmus (24) et secondement la disposition de ces bâtonnets qui chez la larve forment une série ininterrompue tandis que chez l'Echinochasmus adulte ils forment une séries interrompue sur la face dorsale du disque adoral.

Si nous considérons que les bâtonnets et les épines des Trematodes sont des formations cuticulaires qui peuvent se réduirent en nombre ou même disparaître pendant le développement de ces animaux, ce n'est pas trop hasarder d'admettre que la larve dont nous avons parlé soit celle d'*Echinochasmus perfoliatus.* Nous avons aussi des exemples: Ainsi Looss a observé chez *Stephanochasmus bicoronatus*—Trématode très proche des Echinostomiidés—que la double couronne de bâtonnets de l'orifice buccale, laquelle chez les exemplaires normaux est interrompue sur la face ventrale, chez d'outres exemplaires cette double couronne est complétée par la présence d'un nombre supplémentaire de bâtonnets au lieu de 31. D'ou cet auteur tire la conclusion très logique que le type primitif de *Stephanochasmus bicoronatus* aurait eu un plus grand nombre de bâtonnets autour de l'orifice buccale, qui se seraient réduits avec le temps, et que les bâtonnets supplémentaires

doivent être considérés comme des retours à l'état primitif. De même Kobayashi chez *Clonorchis sinensis* et moi chez *Opisthorchis felineus* nous avons montrés que les larves de ces Distomes ont des épines disparaissant complètement pendant leur dévelopment jusqu'à l'adulte, ce qui dénote que le type primitif de Clonorchis et Opisthorchis a eu des épines tégumentaires.

Vu ces exemples on peut supposer que le type primitif d'Echinochasmus ait eu un disque adoral avec 27 bâtonnets et que ce nombre serait réduit avec le temps par la disparition de trois bâtonnets de la face dorsale du disque adoral, et que ce sont seulement les larves qui ont conservé le nombre et la disposition de bâtonnets du type primitif d'Echinochasmus.

Il est intéressant de mentionner qu'Ercolani croyait avoir reproduit expérimentalement l'*Echinostomum echinatum* Zeder, parasite de l'intestin, de la poule et quelques oiseaux aquatiques, chez le chien en lui faisant ingéré *Cercaria echinata* (Sieb.) ; Railliet et Henry sont d'avis que ce Echinostome était *Echinochasmus perfoliatus*. Moi, je suis sûr que ce ne sont pas les mollusques, qui ont infesté le chien d'Ercolani avec cet Echinostome, mais que le chien était infesté avant l'expérience par l'ingération de quelques poissons.

On fait encore mention dans la littérature sur la présence chez les poissons marins de larves d'Echinostomes. Ainsi Stossich nous dit avoir trouvé dans la cavité abdominale de *Gobius jazo* l'*Agamodistoma valdeinflatum* (Stossich) qui représenterait d'après lui la larve d'*Echinostoma cesticillus* de l'intestin de quelques poissons de mer. Fiebiger a trouvé une larve semblable à l'*Agamodistoma valdeinflatum* dans des tumeurs de la peau de *Zeus faber*. Mais nous savons qu'actuellement on place les Distomes du type d'*Echinostoma cesticillus* dans le genre *Stephanochasmus* qui forme un groupe à part voisin de la famille des Echinostomiidés.

Il me parait que Yokogawa dans les dernières années à l'occasion de ses recherches sur les larves de *Metagonimus yokogawai* aie trouvé dans les branches d'une Forelle (*Plecoglossus altivelis*) parmi les larves de Metagonimus encore des larves d'Echinostomes, mais qu'il ne les reconnut pas. D'après Yokogawa ces larves seraient plus petites que celles de Metagonimus, elles sont incapsulées dans des chistes elliptiques qui mesurent 0.105-0.119 mm. de longueur et 0.056-0.07 mm. en largeur. La ventouse orale de ces larves est armée de petites épines. Le corps est immobile et avec une structure confuse. De ces larves on n'a pu déterminer chez les animaux d'expériences le ver adulte.

Quoique la description donnée par Yokogawa à ces larves est incomplète, je crois qu'elles représentent des larves d'Echinostomes.

Le fait qu'en Asie orientale l'infection de l'homme avec *Clonorchis sinensis* et *Metagonimus yokogawai* a lieu par la consommation des poissons crus et que chez ces poissons on pourrait trouver des larves d'Echinostomes, m'a suggéré l'idée qu'aussi l'infection de l'homme aux Philippines par *Echinostomum ilocanum* et à Malacca par *Echinostomum malayanum* serait produite par la consommation des poissons crus. En effet Garrison et Leiper ont recueilli ces Echinostomes de l'intestin des indigènes qui sûrement mangent les poissons ainsi que les Japonais à l'état cru.

Cette supposition trouve sans ancun doute encore un point d'appui dans le fait que j'ai pu déterminer expérimentalement que l'infection du chien et du chat avec *Echinochasmus perfoliatus* est produite par la consommation de quelques poissons crus.

Bibliographie

Motas, C., si Straulescu, N. 1902.—Distomatosa intestinala a câinelui. Bul. soc. Med. Veter., Sedinta ord. 5 Decembrie.

v. Ràtz, St. 1908.—In Fleischfressern lebenden Trematoden. (Külonlenyomat az Allattani Közlemenyek. Kötétenek 1, fuzeteböl. 7:16-20; fig. 1-2. [En hongrois avec un résumé en allemand.])

Railliet, A., et Henry, A. 1909.—Sur un Echinostome dans l'intestin du chien. Compt. rend. soc. biol., 66: 447.

Ercolani, G. B. 1881.—Dell'adattamento della specie all ambiente. Mem. Acc. sci. ist. Bologna, 2: 86 (Cité d'après Railliet et Henry).

Stossich, M. 1908.—Saggio di una Fauna Elmintologica di Trieste e Provincie Contermini, 1: 59.

Fiebiger, Y. 1908.—Ueber durch Trematoden verursachte Hautwucherungen bei Zeus faber und das subkutane Vorkommen von Trematodencysten. Centralbl. Bakter. (Orig. 1), 47: 62-69; 2 fig.

Looss, A. 1901.—Ueber die Fasciolidengenera *Stephanochasmus, Acanthochasmus* und einige andere. Centralbl. Bakter. (Orig. 1), 29: 595-661; 14 fig.

Kobayashi, H. 1915.—On the Life History and Morphology of *Clonorchis sinensis.* Centralbl. Bakter. (Orig. I), 75:299-318; 38 fig.

Yokogawa, S. 1913.—Ueber einen neuen Parasiten *Metagonimus yokogawai,* der die Forellenart *Plecoglossus altivelis* (Temminck) zum Zwischenwirt hat. Bildung einer neuen Gattung. Centralbl. Bakter. (Orig. I), 72: 158, 17 fig.

Ciurea, J. 1914.—Recherches sur la source d'infection de l'homme et des animaux par les Distomes de la famille des Opisthorchiidés. Bull. sec. sc. Acad. Roumaine (7), 2: 201-205.

1915.—Weitere Versuche über die Infektionsquelle des Menschen und der Tiere mit Leberdistomen aus der Familie Opisthorchiiden. Zeitschr. Infektionskr., parasit. Krank. u. Hyg. d. Haustiere, 17: 209-214; 3 fig.

1915a.—Un Echinostome dans l'intestin du porc. Centralbl. Bakter. (Orig. 1), 74: 392-394; 7 fig.

1917.—Die Auffindung der Larven von *Opisthorchis felineus, Pseudamphistomum danubiense* und *Metorchis albidus,* und die morphologische Entwicklung dieser Larven zu den geschlectsreifen Würmern. Zeitschr. Infektionskr., parasit. Krank. u. Hyg. d Haustiere, 18: 301-357; 21 fig.

ON THE STRUCTURE OF SOME MICROSPORIDIAN SPORES *

R. Kudo

The structure of the spores of Microsporidia has been variously described by several authors for different species. Even in one and the same species, the observations of many investigators do not seem to agree. This controversy may be attributed partly to the minuteness of the object and partly to the difference in the technic used. On examining the numerous papers on Microsporidia, one would be impressed by the fact that the majority of the authors do not state their observations with positiveness.

A more or less generally accepted conception of the structure of the Microsporidian spore seems to have been given by Mercier (1908) for *Thelohania giardi* (length 5 to 6μ, after Thélohan). Mercier observed that the spore is covered with a bivalve shell, each valve developing from an uninucleated parietal cell, that the spirally coiled polar filament is contained in a polar capsule with a nucleus, that the girdle-shaped sporoplasm with at first two, later four nuclei, surrounds the polar capsule, and that a vacuole is present at each pole of the spore. This view, on the whole, has been confirmed by Schröder (1908), Stempell (1909), Fantham and Porter (1912, 1914), Strickland (1913), Kudo (1916), and others, altho their observations differ in details.

On the other hand, Schuberg (1910) noticed in the spores of *Plistophora longifilis* (macrospore, 12μ long, 6μ wide; microspore, 3μ long, 2μ wide) that the girdle-shaped sporoplasm which is circular in cross-section, contains a single nucleus, that the polar filament is coiled directly under the shell mostly in the posterior portion of the intrasporal space, that the so-called polar capsule does not occur in the Microsporidian spore, and that the nuclei observed by other authors, are none others than the metachromatic granules. The same view has been maintained by Omori (1912), Weissenberg (1911, 1913) and Debaisieux (1913, 1915).

Léger and Hesse (1916) described an interesting type of Microsporidia under the generic name of Mrazekia. The spores are of cylindrical or tubular form, and show an entirely different structure compared with other genera. The polar filament is differentiated into two parts. No polar capsule is mentioned as present, the polar fila-

* Contributions from the Zoological Laboratory of the University of Illinois, No. 151.

ment being coiled directly inside of the shell. Instead of being in form like a girdle, the binucleated sporoplasm is a rounded and more or less well defined body embedded in a clear space at the posterior portion of the spore.

The same authors (1916a) later reported a similar observation made on the structure of the spore of *Plistophora macrospora* (8.5μ long, 4.24μ wide, after Cépède). They mentioned that the polar capsule lies closely to the shell, occupying the greater part of the intrasporal space, that the polar filament is coiled in the capsule without a central axis, that the sporoplasm is a rounded binucleated body embedded in the posterior vacuole of the spore, that the girdle-shaped structure which was thought to be the sporoplasm by numerous authors is none other than the retracted substance composing the polar capsule so that one or two turns of the polar filament were mistaken in optical cross-section as a variable number of nuclei, and that the granule in the posterior vacuole which was designated as a metachromatic granule by some authors, is none other than the nucleus of the true sporoplasm. Georgévitch (1917) agreed with the above mentioned view in his study on *Mariona marionis,* altho he noticed that the polar capsule was entirely absent in some spores.

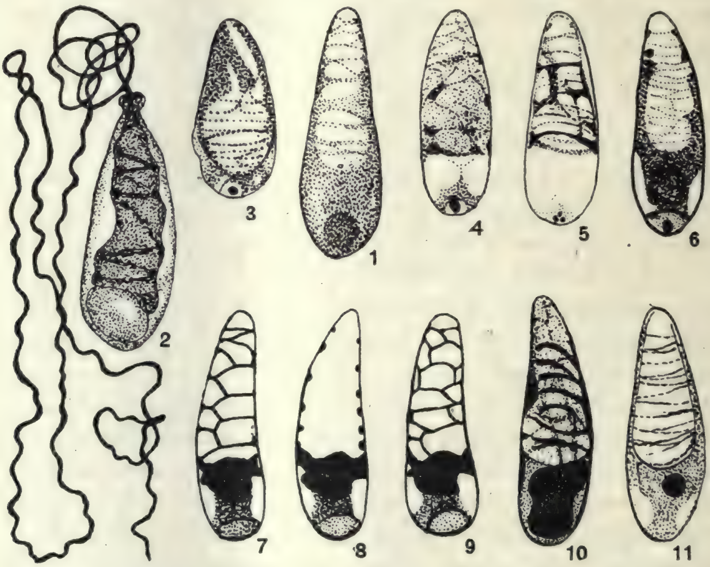
The writer has recently obtained four new forms of Microsporidia from the vicinity of Urbana, Illinois. As their occurrence is rather rare and their life history is now being studied by both natural and artificial infections, it will take some time to complete the work.

One of the parasites, which is a rare parasite in the larvae of *Culex pipiens* from a limited water area in Urbana, proved to be well fitted for the study on the structure of the spore. The spore is from 12 to 13.5μ in length, and 4μ in breadth as measured in stained materials. It is, therefore, one of the largest microsporidian spores that have been recorded. Altho some of the spores of *Plistophora longifilis* and *Thelohania legeri* have the same length, the majority of the spores of these two species are small, while the present form has its advantage in having spores of uniformly large dimensions. Besides, the shell is very thin so that the internal structure could, to some extent, be made out in vivo.

The spore is elongated pyriform usually slightly bent toward one side. It is circular in cross-section. The posterior end is broadly rounded, while the anterior extremity is less rounded, tho not attenuated.

In the fresh state, the spore exhibits marked vacuolation thruout the intrasporal space. For about two-thirds of the anterior portion, a fine polar filament coiled like a network can be seen (Fig. 1), which becomes more distinctly visible when stained, while the posterior one-third is occupied by a finely granulated protoplasmic mass which often

contains a refringent body near its extremity. When fresh spores are subjected to mechanical pressure (Kudo, 1913), and stained by Fontana's method, the extruded polar filament is distinctly recognizable (Fig. 2). This filament is uniformly thick, shows usually a wavy course, and reaches a length of 230μ. The writer does not think this as an average length but records it here as the longest one found so far. In Figure 2 is shown not only the extruded polar filament, but also its remaining part coiled spirally inside of the capsule. The same figure gives at the same time strong evidence for the presence of a particular polar capsule with its polar filament. The shell does not



Spores of *Thelohania magna* nov. spec. × 2360. Fig. 1. A fresh spore. Fig. 2. A spore mechanically pressed, and stained after Fontana. Fig. 3. A young spore stained with Giemsa's stain. Figs. 4-6. Spores stained with Giemsa's stain. Figs. 7-9. Three different views of a single spore stained with Giemsa's stain. Fig. 7. The lower surface view. Fig. 8. The optical section. Fig. 9. The upper surface view. Fig. 10. A spore somewhat deeply stained with Giemsa's stain. Fig. 11. A spore stained with Delafield's hematoxylin.

exhibit any sutural line that might suggest a bivalve nature such as one sees in a Myxosporidian spore either in fresh or stained preparations.

When fixed with Schaudinn's fluid, and stained with Giemsa's stain followed by acetone dehydration, Heidenhain's iron hematoxylin, or Delafield's hematoxylin, the spore shows its various structures very distinctly. Inside of the shell, a large pyriform polar capsule becomes more visible together with the polar filament. The polar capsule, 7.5μ in length, occupies about two-thirds of the anterior portion of the

spore as studied in the fresh state. The foramen of the capsule can not be seen clearly, but the fact that the polar capsule opens at the anterior tip of the spore is distinctly shown in Figure 2. The wall of the polar capsule is comparatively thin, and is very faintly stained in many spores treated with Giemsa's stain (Figs. 4, 5, 7-9). In spores stained deeply with the same stain, however, the polar capsule is recognizable as a reddish colored sack (Fig. 10). In younger spores it is well seen (Fig. 3). It is distinctly recognizable when the spore is brought under the influence of mechanical pressure (Fig. 2). A polar capsule of similar appearance was observed by Schrröder (1914) in *Thelohania acuta,* altho the same author did not trace out the filament. The polar filament is coiled spirally along the inner surface of the polar capsule. Its spiral course begins at the anterior tip of the capsule, and does not differentiate a central axis, altho some longitudinal courses were often seen in the posterior portion of the capsule (Fig. 10). Figure 3 shows the developing polar filament in a young spore; the windings are more or less clearly visible. In a deeply stained spore, the spiral can be recognized distinctly (Fig. 10). Three different views of a single spore treated with Giemsa's stain are shown in Figures 7 to 9, which exhibit the spiral course more distinctly along the inner surface of the polar capsule than any other spores. The spirality of the present form is, therefore, somewhat similar to that of *Plistophora macrospora* (Léger and Hesse, 1916a), of *Nosema bombycis* (Kudo, 1916), and of most of the Myxosporidian spores (Auerbach, Kudo, Davis, etc.); but differs from Stempell's (1909) observations on *Nosema bombycis* and from Mrazekia studied by Léger and Hesse (1916).

The rounded sporoplasm occupies the posterior third of the spore. In fixed preparations a clear space is seen on its lateral side (Figs. 6, 7-9, 11). The nucleus is a comparatively large rounded compact mass embedded in the sporoplasm, and shows typical nuclear staining by the above mentioned stains. It is well differentiated in spores stained with Delafield's hematoxylin (Fig. 11). In every spore stained less deeply with Giemsa's stain the nucleus is represented by a single, or two or three smaller chromatic granules situated regularly at the posterior tip of the spore (Figs. 3-6). No nucleus for the polar capsule or the shell has been recognized. Schuberg (1910) noticed a similar fact in *Plistophora longifilis* as was stated before.

The other three forms have spores of much smaller dimensions, and so far have not shown any fact regarding their structure other than the observations which were presented by the present writer in his paper on *Nosema bombycis* (Kudo, 1916).

SUMMARY

The spore of *Thelohania magna* nov. spec. is of exceptionally large dimensions. Microsporidian spores are not so similarly built as those of different genera of Myxosporidia. A diversity in the structure of Microsporidian spores is recognized with at least two categories: one type, *Nosema bombycis* and the other type, *Thelohania magna*. The latter has a distinct polar capsule with spirally coiled polar filament without central axis; it has a rounded sporoplasm containing a single nucleus. Combination of mechanical pressure and Fontana's staining is especially favorable for the study of the extruded polar filament, and also some structures in the spores. To this type may belong *Thelohania acuta, Plistophora elegans* and *P. macrospora*.

It is interesting to note that altho the parasite attacks only the adipose tissue of the host, infected larvae die more rapidly in captivity than normal ones. So far pupae and adults have been found to be free from infection, which suggests a fatal effect of the parasite upon the host body.

WORKS CITED

Kudo, R. 1916.—Contributions to the Study of Parasitic Protozoa. I. On the Structure and Life History of *Nosema bombycis* Nägeli. Bull. Imp. Seric. Exp. Stat., Tokio, 1: 31-51.

Léger, L., and Ed. Hesse. 1916.—Mrazekia, genre nouveau de Microsporidies à spores tubuleuses. C. R. soc. biol., 79: 345-348.

1916a.—Sur la structure de la spore des Microsporidies. C. R. soc. biol., 79: 1049-1053.

Mercier, L. 1908.—Sur le development et la structure des spores de *Thelohania giardi* Henneguy. C. R. acad. sci., 146: 34-38.

Schuberg, A. 1910.—Ueber Mikrosporidien aus dem Hoden der Barbe und durch sie verursachte Hypertrophie der Kerne. Arb. kais. Gesundh., 33: 401-434.

OBSERVATIONS ON ABNORMAL COURSES OF INFECTION OF *PARAGONIMUS RINGERI* *

Sadamu Yokogawa and Susumu Suyemori

It is a well known fact that infection with *Paragonimus ringeri* is caused by the swallowing of the encysted larvae. It is, however, important to know whether these larvae, if freed from their cysts, can infect through the skin, mucous membrane or any wound on the skin of the host. It seems that this might be possible since they can penetrate the intestinal wall, diaphragm, connective tissues, muscles and some of the viscera of the host after the encysted larvae are swallowed. To test the possibility of such infections, we carried through experiments in an attempt to answer the following questions.

1. Can the freed larvae penetrate the sound skin of the host? The larvae after being freed from their cysts are injured in fresh water, and lose their power of movement, so that some other medium was necessary for the experiments. They are more active in artificial intestinal juice or in normal saline. To determine whether active larvae can penetrate the skin of the host under suitable conditions we experimented on mice, cats and new-born puppies. The results of these experiments are as follows:

(a) Although the freed larvae move actively in the artificial intestinal juice or normal saline, yet they can not penetrate the sound skin of the mice and puppies in a room temperature below 30° C., since their movement decrease below 37° C.

(b) We stretched three mice on a small plate after shaving off the hair of the abdominal wall and dropped upon them the artificial intestinal juice containing the freed larvae. We then put them into a warm chamber at 38° C. After one and a half hours we found that in one case there was a slight desquamation on the abdominal wall, but we could find no evidence of penetration by the larvae.

These experiments prove that the freed larvae cannot penetrate through the sound skin of the host.

2. Can the freed larvae infect through a wound in the body? It is conceivable that the freed larvae might penetrate a wound on the body. It is necessary, however, to try by experiment whether they can actually infect from an exposed wound of the host. On July 15, 1918, we dropped the normal saline with many freed larvae on fresh wounds, which we made in the backs of two dogs. After awhile we found

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* From the Pathological Department of the Medical College of Formosa and the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

several points of hemorrhages caused by the perforation of these larvae. Then we covered the wound with a watch glass and bound it to the body in order to prevent infection from the licking of the wound. One of the dogs died on August 20, thirty-five days after the experiment, and the other on September 10, having passed fifty-seven days after the experiment. By dissection, we could not find any distomes in the first dog, but in the last case two freed distomes were discovered in the chest cavity.

By these experiments we proved that occasionally the freed larvae can infect a host from a fresh wound on the body.

3. Can the freed larvae infect the host through the mouth? In Korea, R. Kawamura *(Tokyoer med. Woch. No.* 1986, 1916*)* proved experimentally that *Paragonimus ringeri* which is growing in its final host, can continue development after being fed to other animals. This mode of infection is very interesting in relation to lung distome disease, because many Koreans have the habit of eating the meat and the liver of dogs and other animals raw. If such animals had the young distomes wandering in their muscles, connective tissues and liver, this habit might lead to the infection of man. Therefore, we examined this point very carefully, using seven dogs, with these results:

(a) A new-born puppy was fed with 25 larvae, which were just freed from the cysts in the artificial intestinal juice, on July 31, 1917. It was killed on October 2, sixty-one days after feeding. On dissection, we found a wormcyst in the middle lobe of the left lung, in which were two distomes, and a freed distome in the left pleural cavity.

(b) A new-born puppy was fed with 25 larvae which had just been freed from their cysts, on Aug. 2, 1917. The puppy died on August 23, having passed twenty-one days after feeding. On dissection the next day, we found a distome in its right chest cavity, and numerous ankylostomes and ascarids which caused the death of the dog.

(c) A young dog was fed on Dec. 9, 1916, with 15 distomes which had lived for forty-two days in another dog, and killed on the twenty-fifth of the same month, fourteen days after feeding. On dissection we could not find any worms in the body.

(d) A young dog was fed on Aug. 19, 1918, with 20 distomes, which had lived for forty-two days in another dog. The dog died on the thirtieth day of that month, ten days after feeding. On dissection, we could not find any distomes in its body.

(e) A young dog was fed on July 8, 1918, with 27 distomes, which had lived for fourteen days in another dog, and 51 distomes on the thirty-first of the same month, which had lived for twenty days in another dog. Next day we killed this dog, five days having passed after the first and one day after the second feeding. On dissection, an hemorrhage was observed in the intestinal wall. This hemorrhage

probably was caused by the action of the distomes, therefore we looked for the worms with special care, but could not find any worms in the body.

(f) A young dog was fed on June 15, 1918, with 18 distomes, which had lived for eighteen days in another dog, and on the nineteenth of that month with 9 distomes which had lived for twenty-three days in another dog. On June 21 it was killed, having passed six and two days after first and second feeding. Careful dissection failed to disclose any worms.

(g) A young dog was fed on June 10, 1918, with 228 larvae just freed from their cysts and killed twenty hours after feeding. On dissection, some inflammation was observed here and there on the serous membrane of the viscera, and many hemorrhages were found in the intestinal wall. It is evident that these hemorrhages were caused by the perforation of the distomes, because we found 21 distomes in the abdominal cavity. The diaphragm and the lungs were intact. There were no distomes in the pleural cavity.

From these experiments we learned that the larvae which were just freed from the cysts as well as the encysted larvae can infect by way of the mouth, but that distomes which were in a more advanced stage of development in their host, cannot develop after feeding to other animals. This indicates that the worms, which are partly developed in one host, find it difficult to pierce the wall of the intestine when introduced into another host, because of the decrease in activity which comes with growth. We proved this fact by using the mucous membrane of lips and the conjunctiva of dogs. For example, if we put some newly freed larvae on the conjunctiva, or on the mucous membrane of the lips of a dog, they soon penetrate the mucous membrane, but the distomes, which are partly developed in one host, cannot bore through these membranes. While our experiments showed that partly grown larvae of *Paragonimus ringeri* could not be transfered from one host to another, two cases of such infection have been reported by Mr. R. Kawamura and by Dr. Ando * (*Rept. Jap. Path. Soc.* v. 6, 1918). On account of these exceptional cases, it will be necessary to forbid the eating of the raw flesh and livers of animals which can harbor the lung fluke.

4. Can the freed larvae infect through mucous membranes outside the digestive tract? To ascertain the pathological changes in the orbits caused by *Paragonimus ringeri,* we dropped normal saline containing larvae just freed from their cysts, on the conjunctiva of dogs,

* Dr. Ando later repeated this experiment, using eighteen white rats. His results in this second experiment failed to show development in a second host of larvae which had lived for a period in one final host (*Tokyoer Med. Wochenschr.*, No. 2163, 1919).

cats and rabbits. In a little while, we found some small hemorrhages, which were caused by the penetration of the worms. In this experiment, nine rabbits, seven dogs and two monkeys were used, and were examined at various times after these experiments. Each animal showed the presence of the distomes after careful examination first of the orbits and then of the whole body. We proved that the distomes which entered into the orbits were to be found in that place for ten or fifteen days after the beginning of the experiments. But in the cases in which twenty days or more had passed after the experiment, they were found in the chest cavity and not in the orbits. It is very interesting to know how they found their way from the orbits to the chest cavity. We demonstrated this point on experimental animals, and will describe the two most interesting cases.

(a) On September 1, 1918, we put 14 distomes into the right, and 17 into the left orbit of a young dog by cutting open the capsule of Tenon. These distomes were collected from a dog, which was fed with a large number of the encysted larvae sixty-one days before. The dog died on the ninth of that month, eight days after the experiment. On dissection, there was found some muddy exudate in the pleural cavity. The lungs were congested a little and showed some irregular points of hemorrhages here and there, but we could not find any pathological changes, caused by penetration of the worms. We found a distome along the vena cava superior, near the lower end, of the trachea, and another distome on the diaphragm of the right side. In the dissection of the neck we found a distome, which was moving in the loose connective tissue of the right side, about the middle of the trachea, and another distome in the submucosa of the posterior wall of the pharynx. Both eyeballs were badly injured by the operation, but we could not find any worms in the eyeballs and in the orbits. We found only a small suppuration, which was due to the boring of the worms, and the subsequent infection by bacteria, in the tissue of the upper corner of the left orbit and in the left temporal muscle.

(b) On October 11, 1918, we put four distomes into the right and fourteen into the left eye of a big dog by the same operation. These distomes were all mature. The dog died on the twenty-fifth of that month, 14 days after the experiment. On dissection, 2 distomes were found; one of them was situated on the bifurcation of the internal maxillary artery and the superficial temporal artery, and the other worm was located in the masseter muscle around the masseter artery. The lungs and the other viscera were intact.

From these experiments we concluded that the distomes which were dropped on the conjunctiva, penetrate into the orbits and live there a certain number of days. Afterward they escape from the orbits and move to the chest cavity, wandering in the soft tissues.

Therefore, in the monkey's orbits, which are enclosed completely by the bones, they remained a very long time. In the case of a monkey, which was examined eighty-four days after the experiment, we found a living distome in the orbit instead of in the chest cavity.

SUMMARY

1. Young active larvae of *Paragonimus ringeri,* just freed from cysts, cannot penetrate the sound skin, but can enter through a fresh wound.

2. Such young distomes can infect the body by the mouth. Their course from the intestine to the lungs is very similar to the route taken by the encysted larvae.

3. Young distomes just freed from cysts can penetrate the mucous membrane outside of the digestive tract, like the conjunctiva, and bore through the tissues until they reach the lungs.

4. Our experiments indicate that other animals cannot be infected by distomes, which had started development in the final host.

5. Partly developed distomes cannot penetrate the mucous membrane, but, if transferred to the orbits of a suitable host, they penetrate the tissues and reach the lungs.

A NEWLY DISCOVERED PARASITIC NEMATODE

(*Tylenchus mahogani,* n. sp.)

CONNECTED WITH A DISEASE OF THE MAHOGANY TREE

N. A. Cobb
United States Department of Agriculture

Tylenchus mahogani, n. sp. $\frac{3.4}{3.7}\cdots\frac{15.}{4.8}\cdots\frac{21.}{4.8}\cdots\frac{{}^{68}\text{—}83\text{-}{}^{5}}{4.1}\cdots\frac{96.3}{2.3}\ 0.56\ \text{mm}$ The naked transparent, colorless cuticle is traversed by about one thousand plain transverse striae to the millimeter. The striae are more or less easy of resolution, and, owing to their presence, the plain contour of the body becomes more or less crenate on the tail of the female. Two wings occur opposite each lateral field, consisting of two double lines, beginning near the head and ending near the anus, and occupying a space one-third as wide as the body. There are no longitudinal striations in the cuticle, but the attachment of the somatic muscles gives rise to faint longitudinal markings in the subcuticle. The rounded or subtruncate continuous head presents a mouth-opening of the usual character—not at all, or exceedingly little, depressed. The minute lips are thoroughly amalgamated, and present a refractive, six-ribbed framework as the support of a flattish dome very much like that found in related species, such as *Tylenchus musicola.* The lip-region may be described as being flattish hemispherical, and its framework may appear as if made up of a series of six loop-like elements. The tubular pharynx is entirely typical. The refractive spear, which is about as long as the base of the head is wide, is a rather prominent feature. Its base is faintly trilobed and one-fifth to one-sixth as wide as the base of the head. The main shaft of the spear, comprising the posterior half, is one-half as wide as the bulb, and its junction with the tapering anterior half of the spear is marked by a distinct, but exceedingly fine, encircling ridge. The guiding pieces for the spear, forming the axial part of the lip-region and surrounding the spear, are three-fifths as long as the tapering anterior half of the spear. Posteriorly, the neck is more or less cylindroid; anteriorly, it is conoid. No traces of amphids or eye-spots have been seen. The subspherical median bulb of the esophagus is one-half as wide as the corresponding portion of the neck, and is set off by a constriction in front. The anterior portion of the esophagus tapers more or less to the median bulb, so that at the base of the pharynx the esophagus is two-fifths, at the nerve-ring one-fifth, as wide as the corresponding part of the neck. The lining of the esophagus is a distinct feature. Esophageal glands of the usual tylenchoid character appear to be present. The median bulb contains a sub-spheroidal valve one-fourth as wide as

itself. The intestine, which, as is often the case in Tylenchus, begins in a rather indefinite way, has a faint lumen and thick walls, and in cross-section presents few cells. There is no pre-rectum. The rectum is inconspicuous and about as long as the anal body diameter. The continuous anus is also inconspicuous. The granules packed in the cells of the intestine are of variable size, the largest being about one-eighth as wide as the body; they do not give rise to a tessellated effect. The straight tail of the female is conoid to convex-conoid, and tapers from the anus, or from somewhat in front of the anus, to the subtruncate or rounded, symmetrical terminus, which is two-thirds to three-fifths as wide as the base of the tail. There is no spinneret, and there are no caudal glands. There is a single lateral innervation on each side near the middle of the tail. The longitudinal fields are three-fifths as wide as the body. The excretory pore lies near the nerve-ring, which is oblique and of medium size. There is a posterior, rudimentary branch to the female sexual organs, about half as long

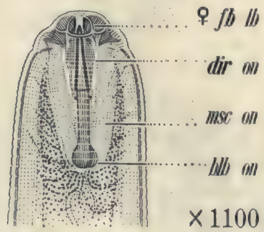

Fig. 1.—Lateral view of head of female of *Tylenchus mahogani, n. sp.* *fb lb,* framework of lip region; *dir on,* guide for spear; *msc on.* protruding-muscle of spear; *blb on,* bulbous base of spear.

as the body-width, and one-half as wide as long. From the well-developed elevated vulva, the large cutinized vagina extends inward at right angles to the ventral surface of the body. The uterus and ovary are outstretched toward the neck. The uterus is of such a size as to contain but one egg at a time. The uterine egg appears about twice as long as the body is wide and half as wide as long, and is covered by a thin, smooth shell. The eggs are deposited after segmentation begins; in fact, frequently contain embryoys. The medium-sized ovary is tapering in form, and near its blind end is only about one-third as wide as the body. It contains up to about twenty ova, which in the principal part of the ovary are arranged single file, but towards the blind end are arranged more or less irregularly.

Male: $\frac{3.2}{3.2}\ \frac{13.}{3.7}\ \frac{19.}{4.}\ \frac{\overset{67}{-M}}{4.4}\ \frac{94.8}{2.8}$ 0.52 mm. The two, equal, colorless, rather strong though slender, subarcuate spicula, at their widest part are one-fifth to one-sixth as wide as the corresponding portion of the body. They taper distally to a sub-acute apex. They are one and

one-half times as long as the anal body-diameter, are cephalated by a broad and shallow constriction, and are so arranged that their proximal ends appear to lie more or less dorsad from the body axis. The single, straight, or slightly arcuate, rather slender, more or less frail, simple, non-apophysate accessory piece lies parallel to the spicula and is about one-third as long as they. There are no supplementary organs. The rather plain-margined bursa springs from opposite the heads of the spicula at a distance in front of the anus as great as the corresponding body diameter. When seen in profile the somewhat

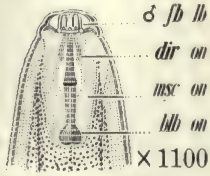

Fig. 2.—Lateral view of head of male of *Tylenchus mahogani, n. sp.* *fb lb,* framework of lip region; *dir on,* guide for spear; *msc on,* protruding-muscle of spear; *blb on,* bulbous base of spear.

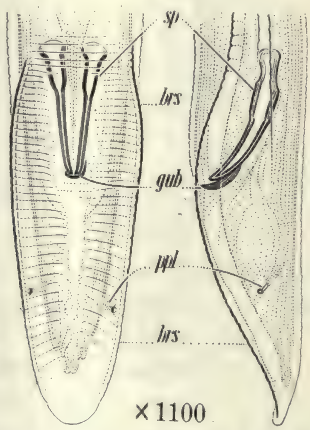

Fig. 3.—Ventral and lateral view of tail end of male of *Tylenchus mahogani, n. sp.* *sp,* spicula; *brs,* bursa; *gub,* gubernaculum or accessory piece; *ppl,* papilla.

rudimentary flaps of the bursa hardly reach the ventral contour. The bursa extends behind the anus a distance twice as great as the anal body diameter, just about encompassing the tail. On each side about midway on the tail, there is an inconspicuous lateral rib or papilla. There really is no very distinct flap to the bursa, so that in the ventral aspect the tail-end of the male appears widened only a very trifling amount, and the ventral surface only slightly concaved. Somewhat in front of the anus the two wings diverge, so that opposite the anus, they occupy two-thirds of the width of the body; here the dorsad

branch ceases, while the ventrad branch continues, and, expanding, forms the bursa, which is rather inconspicuous when seen laterally — rather less so than the illustrations would indicate.

Habitat: Tissues of the mahogany tree. Fixed in formalin, mounted and examined in water. This resembles *Tylenchus musicola* Cobb, but differs in the following respects:

1. It is relatively broader in the ratio of about 4 + : 3.
2. It is less coarsely striated.
3. The bursa is less obvious and not so thin.
4. The spear is more refractive, though relatively not so wide or long.
5. The guiding apparatus to the spear is more obvious.

The species also bears considerable resemblance to *Tylenchus coffeae* Zimmerman.

The following table give comparative data with reference to these species:

Comparisons

	Spear			Bursa
	Length and Width in Terms of Diameter of Base of Head		Nature and Composition of the Spear	
	Length	Width		
T. mahogani n. sp. ...	1	⅕	Refractive. Bulbs amalgamated	Not obvious, thick; not exceeding ventral contour; not exceeding tail
T. coffeae Zimm.	1–1⅕	¼–⅕	Refractive. Bulbs distinct	Obvious, thin; exceeds ventral contour; in length, equals tail
T. musicola Cobb.....	1⅓–1½	⅓	Faint. Bulbs distinct	Obvious, thin; exceeds ventral contour; not exceeding tail

It remains uncertain how serious this disease of the mahogany tree may be. The Director of Agriculture for Barbados, Mr. John R. Bovell, in a letter to the writer, says:

"I have known trees that have shown indications of being attacked for over thirty years which are still alive, and practically no different in appearance than they were when I first noticed them.

"I have been trying to see whether it was not possible to destroy the nemas by taking off the bark and the cambium layer practically down to the young wood of one-half of the attacked portion of the base of the tree and painting it with lime sulphur wash, repeating the painting in about a week and a half, allowing that half of the tree almost to heal before the other half was treated. The tree I treated bore the treatment exceedingly well and did not seem to feel the effects in the slightest, but I do not think we have succeeded in killing all the nemas."

CRITERIA FOR THE DIFFERENTIATION OF SCHISTOSOME LARVAE *

Ernest Carroll Faust

Stimulated by the practical phases of the Schistosome problem, several investigators have recently made notable contributions to the morphology and life-history of the Schistosome group. Cort (1919) has cleared up the details of structure of the cercaria of *Schistosoma japonicum*, while Leiper (1915) and Iturbe (1919) have demonstrated life-histories. Intensive study of this group of cercariae has made possible the ready separation of the human schistosome larvae from those of the group which infect other animals and, at the same time, has allowed a differentiation among the human species.

Thus the cercariae of the human schistosome species lack certain details of structure found in the non-human schistosome larvae. As far as the present knowledge indicates the former have no fluted margin to the tail trunk or furcae. They lack eye-spots. They have no pharyngeal sphincter around the esophagus. Moreover, they possess a smaller number of flame cells than is found in any other cercaria where the flame-cell formula is known.

The human schistosome cercariae have in common an integument covered in its entirety with heavy spines, usually somewhat heavier at the anterior end than near the furcal tips. They have a large pyriform oral sucker directed anteriad, but opening somewhat ventrad as Cort (1919) has shown. In the case of the cercariae of *Schistosoma haematobium* and *S. mansoni* the orifice is larger than in the cercariae of *S. japonicum* and is not conspicuously ventrad (see figures).

A conspicuous organ in the anterior region of the cercaria of the Japanese fluke is the head gland. I have found it to be decidedly basophilic in reaction, resembling the basophilic mucin glands of the larva of *S. mansoni*. Unlike this species, the other two species lack such an organ, by which means they may be readily separated from it. All three species have large conspicuous unicellular mucin glands with heavy ducts which open thru hollow boring spines anteriad and laterad with respect to the mouth.

Cort (1919: 500) describes these glands in detail for the cercaria of *Schistosoma japonicum*. There are five of them on each side with ducts passing ventrad to the nervous system and hence thru the heavy muscular region of the mouth. More recently he has discovered in

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* Contributions from the Department of Pathology, Union Medical College, Peking, China. Read before the Section of General Medicine, China Medical Missionary Association Conference, Feb. 24, 1920.

his material what I have made out for the three known human species as well as for several non-human forms, that each duct is capped by a hollow boring spine, so that digestion of the host tissues and boring into them are performed by the same organ (Faust, 1919). These glands in the cercaria of *S. japonicum,* according to both Cort's and my observations on living material, are large organs with granular acidophilic cytoplasm and large nuclei with basophilic reaction. Thus with hematoxylin-erythrosin technic the cytoplasm stains a decided pink, while the nuclei take on a deep alkaline stain.

While probable differences in size and shape obtain in the case of the cercariae of *Schistosoma haematobium* and *S. mansoni,* which allow of their differentiation from the cercaria of *S. japonicum,* the number and type of the mucin glands is the most dependable basis of diagnosis. Thus the larva of *S. haematobium* has only three pairs of glands with a corresponding number of ducts opening strictly laterad to the orifice. The glands have small nuclei and give a simple acidophilic reaction. On the other hand, the larva of *S. mansoni* has six pairs of glands, with an equal number of ducts which are arranged around the orifice dorsolaterally in the form of two compressed crescents. Moreover, this species has the glands differentiated into two types. Two of the glands are acidophilic with large nuclei, while four give a basophilic reaction and have small nuclei. The basophilic glands take on a deep reddish hue with Best's calcium-ammonium-carmin stain. That the content of the gland undergoes a change as it passes into the duct is apparent from the fact that the granules around the nuclei of these basophilic cells are glycogeniferous, while the content of the duct gives a pure mucin reaction.

This method of distinguishing between these species of larvae makes it possible to diagnose two species in material which Dr. F. G. Cawston has sent the writer from Natal, namely, cercariae of *Schistosoma haematobium* and those of *S. mansoni.* The latter species corresponds both by structural and microchemical tests to Iturbe's species for Venezuela. This discovery of the larva of Manson's fluke in Natal is not entirely unexpected after Porter's discovery (1918: 45) of the adult fluke in South Africa. It shows further that the symptoms of rectal and urinary bilharziasis have not in themselves been sufficiently appreciated to discriminate between these parasites. Thus ability to recognize the larvae where a double infection obtains in a certain area adds a valuable check to the diagnosis and the combating of the disease.

A study of the immature larva of *S. mansoni* provided evidence of the fundamental difference between the two varieties of mucin glands in this species. At a stage before the furcae of the tail become

evident (Fig. 6) the two groups have already assumed their relative positions, have divided into the number characteristic of the adult and give the differential staining test.

It seems highly probable that the methods of specific diagnosis found valuable in separating these larval species may be used to equal advantage in other related groups.

References Cited

Cort, W. W. 1919.—The Cercaria of the Japanese Blood-Fluke, *Schistosoma japonicum*, Katsurada. Univ. Cal. Pub. Zool., 18: 485-507, 3 figs.

Faust, E. C. 1919.—Notes on South African Cercariae. Jour. Parasit., 5: 164-175, 1 pl.

Iturbe, J., and Gonzalez, J. 1919.—Quelques observations sur les cercaires de la valée de Caracas. Ier pte. Caracas. 18 pp., 7 pl.

Leiper, R. T. 1915.—Report on the Results of the Bilharzia Mission in Egypt, 1915. Jour. R. Army Med. Corps, 25: 253-267, 15 figs.

Porter, A. 1918.—A Study of the Intestinal Entozoa, both Protozoal and Helminth, Observed Among Natives in Johannesburg, South Africa. Inst. Med. Research, No. 9, 56 pp., 2 pl.

Explanation of Plate XV

Fig. 1.—Ventral view of the cercaria of *Schistosoma haematobium*, showing oral opening, mucin glands and ducts, and germ cells.

Fig. 2.—Lateral view of head of the cercaria of *S. mansoni*, showing relation of mucin duct openings to mouth.

Fig. 3.—Lateral view of head of the cercaria of *S. haematobium*, showing relation of mucin duct openings to mouth.

Fig. 4.—Lateral view of head of the cercaria of *S. japonicum*, showing relation of mucin duct openings to mouth.

Fig. 5.—Ventral view of the cercaria of *S. mansoni*, showing oral opening, mucin glands and ducts, and germ cells.

Fig. 6.—Ventral view of immature cercaria of *S. mansoni*, showing development of mucin glands.

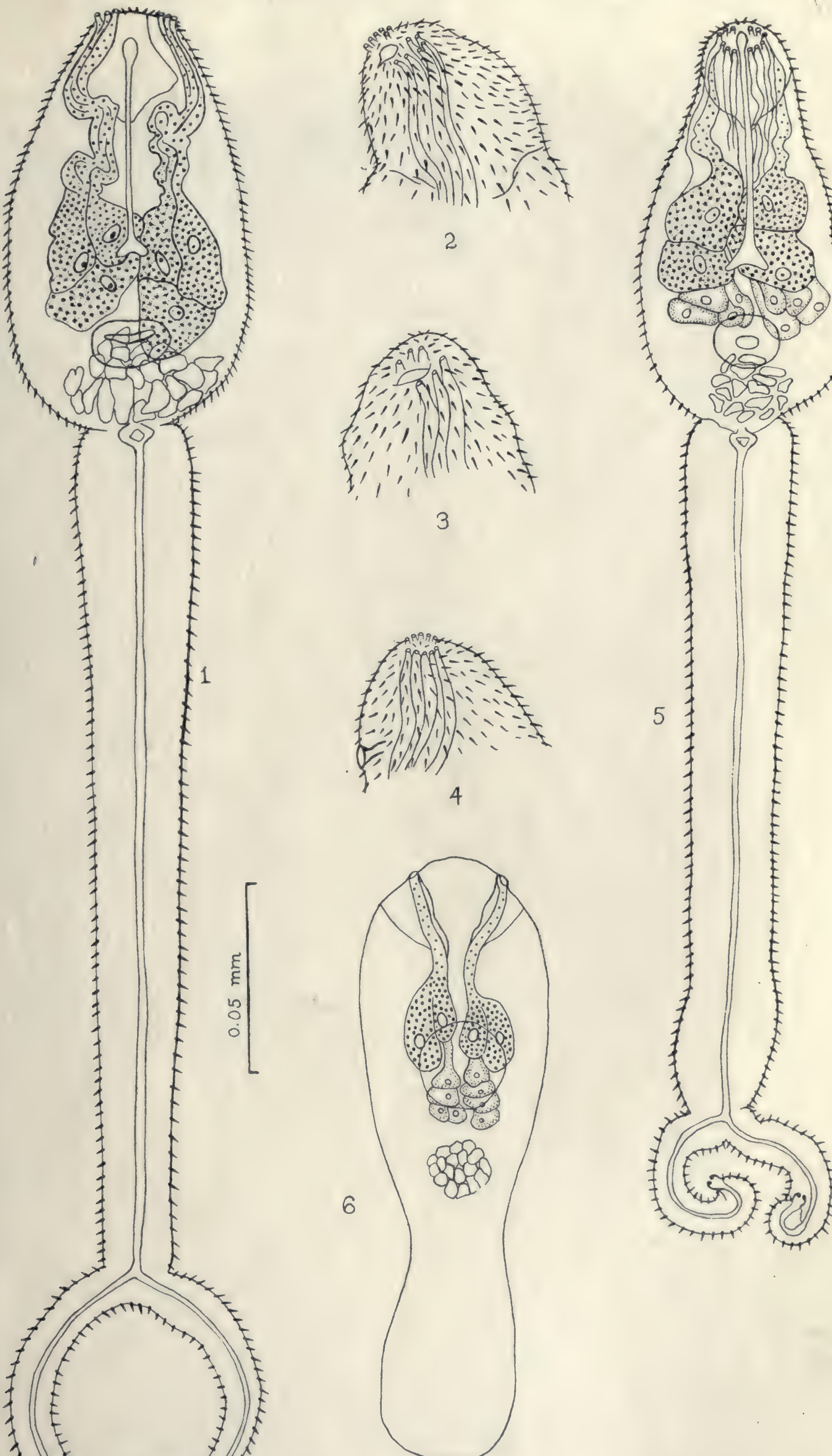


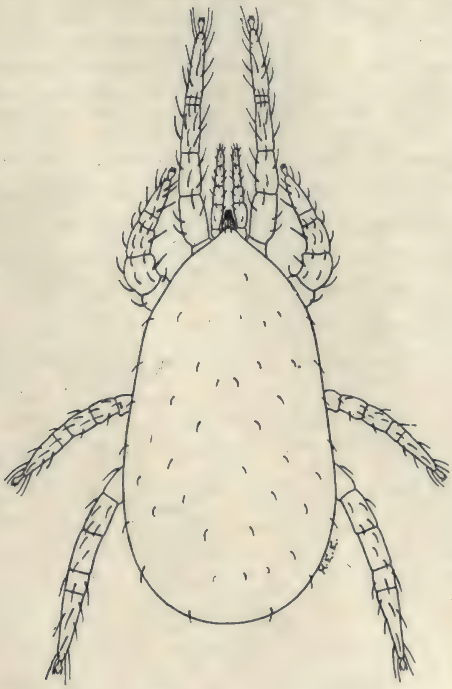
PLATE XV

A GAMASID MITE ANNOYING TO MAN

H. E. Ewing

Bureau of Entomology, U. S. Department Agriculture, Washington, D. C.

On two occasions during the summer of 1919 the writer has encountered a small gamasid mite that gets on the skin of the legs, and for that matter on any part of the body, and causes a considerable annoyance and a "creepy" sensation by running about over the skin. In addition to the annoyance thus occasioned, the mites have a faculty of stopping in the folds of the skin and inserting their mandibles, thus



Hyletastes missouriensis Ewing. Dorsal view, × 110.

causing actual pain. At present it has not been determined whether they engorge blood from man or not. On both occasions these mites were reported as chiggers by other people frequenting the same localities. One of these was along an electric line about five miles west of Washington, D. C., in Virginia, and the other was a front lawn of a friend about two miles from this place. The mite in question was described by the writer in 1909, from material sent to him by Professor

C. R. Crosby, from Columbia, Missouri. It was named *Hyletastes missouriensis.* The material in which the original specimens were contained consisted chiefly of bits of decaying leaves and had been obtained by using a Berlese trap. The mite has also been taken from under bark at Muncie, Illinois. A description of the species is here given:

A small, elongate mite of a uniform, light yellowish, brown color. Sexes alike as far as secondary characters are concerned. Body about twice as long as broad, sides subparallel behind the shoulders, and slightly concave in front of them. The abdomen is broadly and evenly rounded behind. Body all but naked above, yet observed to be very sparcely clothed with small simple setae, a pair of which is situated at the front apex. Ventral abdominal plate circular, slightly over one-half the width of abdomen in diameter; anal plate triangular, one-half as broad as ventral plate. Palpi about half as long as anterior legs and well clothed with setae. Mandibles stout; upper jaw or chela, a stout, projecting, strongly-curved, claw-like hook which surpasses the lower jaw, a short, sharp, curved sword-like, piercing structure; teeth not pronounced, and apparently confined to upper chela. Legs moderate, with rather small claws and ambulacrum. Anterior pair about as long as the body; tarsi about one and a half times as long as tibiae. Posterior legs extending for about one-third their length beyond the tip of the abdomen; tarsi over one and a half times as long as tibiae and divided near their bases. Length, 0.5 mm.; width 0.3 mm.

The potentialities of this species as a pest of man can not be predicted at present because of our lack of knowledge as to its biology and distribution. When it attacks in great numbers it is very irritating. No wheals or discolored spots are produced, hence it is easy to differentiate an attack of these mites from those of chiggers.

ALBERT FRANCIS COUTANT

Albert Francis Coutant was born in Brooklyn, New York, July 7, 1892, and died in Manila, P. I., April 18, 1919. He is survived by his wife, Mary Wotherspoon Stewart, of the Department of Botany of Barnard College.

Dr. Coutant received his B.S. degree from Cornell University in 1913, and his Masters degree in 1914. In 1917 he received his degree of M.D. from the same institution. He was student assistant in Entomology at Cornell from 1911 to 1914, and in the summer of 1912 was assistant in Zoology at the University of Illinois. As an undergraduate his special work in Entomology was largely from the viewpoint of parasitology, and this soon broadened into an interest in the general field.

During the summer of 1916 he worked under the International Health Board of the Rockefeller Foundation on the eradication of the hookworm in Texas. After graduation from the medical college he became established in the Cancer Memorial Hospital in New York, but in September, 1917, accepted an appointment tendered jointly by the International Health Board and the Philippine Health Service to become Chief Surgeon on the Hospital Ship *Busuanga*, operating among the Moros in the southern end of the Philippine Archipelago. During the first six months of 1918 while the ship was undergoing repairs, Dr. Coutant was acting superintendent of St. Luke's Hospital, one of the largest in Manila.

Though devoted to his work, he wrote shortly before his death, "The desire to go back to teaching is still strong with me." The writer happens to know of two tempting university positions in parasitological work which were offered to Dr. Coutant, but he felt that he was under obligations to continue on the hospital ship until the completion of his three year term. This attitude of faithfulness and loyalty was typical of all of his relations in life.

Though his publications were few, he was a keen observer, and accumulated many data which he was planning to utilize in future work. At the time of his death he had several papers in course of preparation, but unfortunately they were not in shape to be completed by another.

Bibliography

Coutant, Albert F. 1915.—The Habits, Life History and Structure of a Blood-Sucking Muscid Larva (*Protocalliphora azurea*). J. Parasitol., 1: 135-150.

1916.—Chenopodium Poisoning. Report of Case. J. Amer. Med. Assn., 67: 1599-1600.

1918.—An Epidemic of Influenza at Manila, P. I. J. Amer. Med. Assn., 71: 1566-1567.

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THIRTIETH TO THIRTY-EIGHTH MEETINGS, 1916-1919

The thirtieth to the thirty-seventh meetings were held at intervals during the years 1916 to 1918. The following includes a few of the papers and notes presented, most of those not reported having been already published or seeming to be no longer of special parasitological interest.

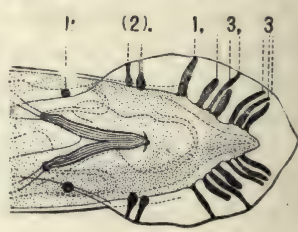
At the thirtieth meeting, March 3, 1916, Doctor Cobb was elected president, and Mr. Crawley secretary.

At the thirty-second meeting, May 12, 1916, Doctor Cobb presented the following note:

BURSAL FORMULA FOR RHABDITIS

As the bursa in this genus is symmetrical, only the papillae and ribs on one side of the bursa are considered, and these are represented by rather arbitrary designations grouped in a formula. These organs are designated according to their proximity to each other and not according to their anatomical and physiological characters. The papillae and ribs are considered as a single longitudinal series, and each group in the series is indicated by a digit representing the number of ribs or papillae in the group. They are regarded as either anal, pre-anal, or post-anal, according as they are opposite to, in front of, or behind the anus. In the formula the anus is included in the parentheses; all papillae approximately opposite the anus are included in the parentheses, the pre-anal papillae are placed in front, and the post-anal papillae after the parentheses. The longitudinal spaces or distances separating the groups of papillae and ribs are

Tail end of a male *Rhabditis*, showing spicula, anal opening, bursa and ribs of the bursa. The ribs of the bursa are shown black. The grouping of the ribs is indicated by the figures above; the corresponding formula as it is to be printed, is shown below the bursa.



1; (2), 1,3,3

indicated by commas and semicolons, the comma representing a short distance, the semicolon a long distance. In some cases before and after the parentheses the punctuation mark may be omitted, thus indicating that the ribs or papillae are even nearer to the anus than in those cases where the separation is indicated by a comma or semicolon. A blank space in the type after the comma, or after the semicolon, indicates a longer distance than is indicated by the comma alone or by the semicolon alone. By these simple means it is easily possible with ordinary type to indicate in a compact formula with considerable accuracy the grouping and latitude of these various elements of the bursa. A glance at the adjacent illustration and formula will make the matter quite clear.

At the thirty-fourth meeting, December 21, 1916, Doctor Stiles gave an account of an investigation made by him and Doctor A. D. Weakley, having for its object to ascertain what connection *Endamoeba gingivalis* has with pyorrhoea alveolaris or Riggs' disease. The study was made on the inmates of the Government Hospital for the Insane, Washington, D. C.

Tests were made with emetin, injected hypodermically daily for six days. In 27 cases, all having endamoeba, the drug was given from July 24 to 31. On

September 22 examinations were made in 25 of these cases with the following results: Marked improvement, 3 cases; slight improvement, 8 cases; no improvement, 14 cases.

The above refers to the conditions as found in the mouth. With regard to the effects on the parasite, 12 showed it 4 days after treatment; 6 showed it 10 days after treatment; 4 showed it 31 days after treatment; 2 showed it 59 days after treatment. In one typical case of Riggs' disease, the amoeba was not present.

The conclusions are to the effect that Riggs' disease is not due to the amoeba and that the amoeba is not always destroyed by emetin.

At the thirty-fifth meeting, January 19, 1917, following a discussion by Doctor Hadwen of investigations on reactions of animals to parenteral injections of juices secured by crushing the bodies of parasites, the results of which have been published elsewhere, Doctor Ransom presented the following note:

REACTIONS FOLLOWING INJECTION OF PARASITE MATERIAL

Experiments have been carried out on the effects of injecting into animals material obtained from various species of metazoan parasites, such as body fluids and aqueous extracts or suspensions of their tissues, either fresh or dried and pulverized. These experiments were suggested by the recent work of Hadwen, of the Canadian Department of Agriculture. In the experiments the host animals used were cattle, horses, sheep, hogs, dogs, cats, rabbits, rats, guinea-pigs, turkeys and chickens, and the parasites included nematodes and tapeworms of various species, ticks, lice, warbles and bots. Few experiments were made on the ophthalmic and intradermal reactions, and the injections in most cases were given subcutaneously, occasionally intravenously. The conclusions reached in some respects are slightly different from those first expressed by Hadwen. Some of the more important are as follows:

Reactions of an anaphylactic type may be produced in cattle, sheep and hogs by single injections of antigens prepared from various metazoan animal parasites.

In some cases the reaction may possibly be specific and dependent upon the existence of infection with the species of parasite from which the antigen is obtained.

In other cases there is no relation between the reaction and the presence or absence of parasites of the species from which the antigen is obtained, and animals may react to parasites of species with which they are not liable to infestation.

Sheep are very susceptible to injections of crushed material, fluids, or extracts from certain metazoan parasites, irrespective of the presence or infestation with these parasites, and small quantities, which have no apparent effect upon guinea-pigs and rabbits when injected intraperitoneally or subcutaneously, when injected subcutaneously into sheep produce severe reactions, frequently terminating in death.

Sheep may respond repeatedly to subcutaneous injections, at intervals of a few days, of material from the same species of parasite, so that the reaction in sheep apparently differs from the ordinary anaphylactic reaction not only in the fact that a sensitizing injection is not required, but in that sheep recovering from one reaction are not thereafter for a considerable period of time insusceptible to further injections.

It is believed that investigations in the field opened up by Hadwen's work will be found to have an important bearing upon the many problems relating to the phenomena of anaphylaxis, and as Hadwen's reaction (that is, the response of animals to antigens prepared from metazoan parasites) in some cases appears to be specific, it may prove of practical utility in diagnosis.

At the thirty-sixth meeting, October 26, 1917, Doctor Ransom was elected president, Mr. Crawley continuing in office as secretary.

Doctor Stiles presented the following note:

A SECOND CASE OF GONGYLONEMA IN MAN

Birge of the Florida State Board of Health has recently seen a case of Gongylonema in a white girl. The case is similar to that recently reported by H. B. Ward. The worm may be either *G. pulchrum* or *G. scutatum*.

The thirty-eighth meeting was held at the residence of Doctor Hall, October 18, 1919. Doctor Ransom was reelected president and Doctor Hall was elected secretary.

Doctor Cobb presented the following note accompanied by a demonstration of the doubly refractive cell inclusions in the intestinal cells of a nematode:

THE USE OF THE POLARISCOPE IN DETERMINING THE CHARACTER OF CELL INCLUSIONS IN NEMAS

In a former paper (J. Parasitol., 1:40-41) read before this society, attention was called to the presence of doubly refractive bodies in the intestinal cells of *Rhabditis monhystera* Bütschli, the name rhabditin being given to the material of which these bodies are composed. In connection with the importation of plants and soil in order to exclude harmful species of nemas that are likely to be present in the small quantities of soil sometimes adhering to the roots of imported plants or in soil brought in as ship ballast, it is important that some broad lines of differentiation be found between harmful and harmless or beneficial nemas, particularly since the imported nemas are commonly of unknown species with unknown food habits. With reference to such a distinction, the granules of the intestinal cells are of interest. As, broadly speaking, the granules are related to the character of the food, the nemas of the two large groups may be expected to show granules of two large general groups. Fortunately, in some nemas food habits are well known. An examination of the intestinal granules of herbivorous nemas and of the less common carnivorous nemas indicates that carnivorous forms that show birefringent granules are approximately twice as numerous as those that do not, whereas the reverse is true for herbivorous forms. Aside from calcium sulphate and rhabditin, five or six kinds of doubly refractive granules have been found in the course of an examination of almost two hundred species of nemas, belonging to about forty genera, and these granules fall into two groups. One of these groups comprises granules that are evidently stored food material, and the other granules that are evidently elimination material; one is anabolic and the other katabolic. The granules of the first group are abundant when present, sometimes comprising more than 25 per cent. of the cell volume. Further study will be made in the hope of distinguishing between herbivorous and carnivorous nemas on the basis of the granules.

Doctor Cobb also presented a note on an adaptation of the polariscope to immersion lenses. In this adaptation the nickel prism is mounted very close to the back lens of the objective. The condenser of the microscope is replaced by an immersion lens, and the object to be examined is mounted between two cover glasses. This apparatus is of great value in studying the cell inclusions in nemas, many of which are on the limits of visibility.

Doctor Ransom presented the following note:

GAPEWORM IN TURKEYS AND CHICKENS

Investigations have shown that the gapeworm of poultry (*Syngamus trachealis*) is found commonly parasitic in turkeys. Feeding experiments show that young chickens readily become infected, but that older birds are comparatively immune, and as a rule cannot be infected by feeding material which is infectious for chicks. At least the worms rarely develop to the mature stage in adult chickens, and when they do succeed in reaching maturity they often

appear to remain in the trachea but a short time, and the chickens soon become free from infestation. On the other hand, adult turkeys can be easily infected as well as young poults, and apparently they can harbor the parasites during long periods. The results of experiments on chickens and turkeys have been confirmed by postmortem observations on birds slaughtered for market purposes. Adult chickens are habitually found free from gapeworm, as shown by an examination of 635 chickens from Center Market, Washington, D. C., all of which were negative. Adult turkeys, however, are commonly found infested. Out of 679 turkeys examined at Center Market, 153, or 22.5 per cent., were infested with gapeworm.

From the foregoing it appears that adult chickens are comparatively of little importance as gapeworm carriers. Adult turkeys, on the contrary, are of major importance as carriers of gapeworms, although they are not likely to be suspected by the poultry raiser as spreaders of infection, since they commonly show no outward symptoms of disease. Turkeys, therefore, must be given consideration as reservoirs of infection as well as the soil in which, according to the results of experiments upon the longevity of gapeworm larvae, infection may persist under favorable conditions for over a year.

Young gapeworms may be found in the lungs within a week after feeding infective material, and the two sexes become coupled in the lungs while still very small. Later they migrate to the trachea, and oviposition begins within two weeks after the feeding of infective material. Gapeworm larvae in guinea-pigs will migrate to the lungs and undergo an incomplete development.

MAURICE C. HALL, Secretary.

BOOK REVIEWS

THE AMOEBAE LIVING IN MAN. A Zoological Monograph. By Clifford Dobell, M.A., F.R.S. New York, William Wood & Company, 1919.

The scientific world is deeply indebted to Doctor Dobell for his recent monograph on the human amoebae. His work, which is truly monographic in character, covers a field that is in a state of serious confusion. To one not intimately familiar with the organisms and unable for any reason whatever to devote much time to the study of previous publications, it is impossible to reach clear conclusions regarding the many questions in dispute. Doctor Dobell has that thorough knowledge which lays the sure foundation for such a study. His long series of valuable studies represented by shorter publications of recent years, the responsibility of training the English workers who devoted themselves to the subject of amoebic dysentery during the war, and membership on the War Office Dysentery Committee made him thoroly familiar with the work done under English auspices. A tireless laboratory worker as well as a keen and impartial critic of the literature, no one else could be named who is anything like as well fitted to give an impartial view of these controversial questions.

Those in any field who are interested in the amoebic parasites of man will find in the volume a work of great interest and helpfulness. After a brief introduction and a useful section on materials and methods, Doctor Dobell reviews concisely the present state of knowledge concerning human amoebae, and then discusses the genera, closing with the following synopsis to indicate the acceptable names and the synonyms:

SYNOPSIS OF GENERA AND SPECIES OF AMOEBAE LIVING IN MAN

Genus I.—*Entamoeba* Casagrandi and Barbagallo, 1895 (nec *Endamoeba* Leidy, 1879).
Synonyms:
Poneramoeba Lühe, 1908.
Löschia / *Viereckia* } Chatton and Lalung-Bonnaire, 1912.
Proctamoeba Alexeieff, 1912.
[*Amoeba* (*pro parte*), *Endamoeba*, *Entamoeba*, *Endameba*, *Entamöba*, Auctt.]
Type: *E. coli* (Grassi) Casagrandi and Barbagallo.
Species in Man: *E. coli* (Grassi) Casagrandi and Barbagallo.
E. histolytica Schaudinn (*emend, Walker*).
E. gingivalis (Gros) Brumpt.

Genus II.—*Endolimax* Kuenen and Swellengrebel, 1917.
Only species, hence type: *E. nana* (Wenyon and O'Connor) Brug.

Genus III.—*Iodamoeba* nov. gen.
Only species, hence type: *I. bütschlii* (Prowazek) Dobell.

Genus IV.—*Dientamoeba* Jepps and Dobell, 1918.
Only species, hence type: *D. fragilis* Jepps and Dobell.

In subsequent chapters each of these genera and species is studied in detail and a complete analysis given of the structure, life history, clinical relations and nomenclature. It would be impossible to review here the immense amount of detailed information compressed into the closely printed pages of the monograph. This section may be commended to the careful study of parasitologists and of clinicians who desire to know the correct form of the names for the various species and the basis on which these conclusions are reached.

Dobell protests rightly against the suppression of the diphthong in the name Amoeba, which has to some extent crept into our literature, probably through the adoption of a quasi-common name ameba. There is certainly no justification for attempting to depart from the Latin language and to modify the original spelling. Dobell also makes it very clear that the genus designation of the human parasite is properly Entamoeba and that the genus Endamoeba, originally

described by Leidy in 1879 for a species of amoebae found in the cockroach, must be preserved for that type.

Some American workers may not look kindly upon the use of the form *Entamoeba histolytica* of Schaudinn in preference to *E. dysenteriae* of Councilman and Lafleur, which has come into use in some circles, but Dobell's argument is unanswerable, and as shown by Stiles some years ago, the name *E. dysenteriae* cannot be justified.

The monograph contains also sections on the amoebae in human urine, in dogs, and in monkeys, as well as on certain other amoeboid organisms described from man which are not true parasitic amoebae of man, but are to be explained in one way or another. They are but a small section of the long list of pseudo-amoebae that could be compiled from the literature of parasitology and medicine.

The work closes with a good bibliography, following which are five plates illustrating the forms under discussion. The colored plates are especially worthy of mention, since they represent in a particularly faithful manner the appearances that present themselves under the microscope to those working with stained and mounted preparations.

The Fifth and Sixth Reports of the Director of Veterinary Research in the Union of South Africa make a splendid volume of scientific contributions in which are some papers of marked interest to parasitologists. Special mention might be made of the work on intoxication by Gastrophilus larvae. It is due to a toxin, but in the view of the authors the symptoms do not accord with anaphylaxis. The life history of a new nematode from fowls (*Filaria gallinarum*) shows developmental stages in termites on which the fowls feed habitually.

NEW HUMAN PARASITES

Monas urinaria Reitler and Robicsek, 1920. — This species of flagellate was observed in the urine in four cases, two of cystitis, one of nephritis, and one of tuberculosis at an army hospital in Vienna. Biflagellate, ameboid, cystic and multinucleate monoflagellate stages are described. The organism was found in the urine only after standing a few hours, and could not be discovered in prostatic or urethral secretions, nor in urine obtained in a sterile condition. The authors therefore conclude that the organism is a free-living form and not a human parasite or commensal (Cent. Bakt., I. Orig., 84:129-132, 1 pl.; Feb. 11, 1920).

Trypanosoma escomeli Yorke, 1920.—Escomel (1919; Bull. Soc. path. exot., 21: 723) described a case of trypanosomiasis from the tropical forests in the eastern portion of Peru. He identified the parasite as probably *Schizotrypanum cruzi,* but Yorke considers that it is probably not of this species because of its larger size (up to 40μ) and because of its small, hardly visible blepharoplast. Accordingly, Yorke proposes the name given above for Escomel's trypanosome. (Ann. Trop. Med. and Parasitol., 13:459-460; March 15, 1920.)

Spirochaeta orthodonta Hoffmann, 1920. *Spirochaeta skoliodonta* Hoffmann, 1920. *Spirochaeta trimerodonta* Hoffmann, 1920.—In an article in which he pays little attention to the rules and customs of zoological nomenclature, Hoffmann discusses various forms of spirochetes that occur in the human mouth, including *Spirochaeta buccalis crassa, S. buccalis tenuis, S. media oris,* and the three others named above. *S. skoliodonta* and *S. trimerodonta* are proposed as new species. *S. orthodonta* is a name proposed as a substitute, apparently in order to secure a sort of uniformity in names, for a species formerly known as *Spirochaeta dentium* or *S. denticola* (Deutsche med. Wchnschr., 46:257-259, 1 fig., March 4, 1920).

Diplocercomonas soudanensis—Because the original generic name was preoccupied, the form noted previously as *Dicercomonas soudanensis* (Jour. Par., 6:48) has been renamed as above by the authors (J. Trop. Med. & Hyg., 22:190; Oct. 15, 1919).

NOTE

Research in the field of Parasitology has suffered a serious loss in the sudden death from infective jaundice of Doctor A. J. Chalmers (*aet.* 50) at Calcutta on April 6. When Doctor Andrew Balfour left Khartoum, Doctor Chalmers became his successor there as Director of the Wellcome Tropical Research Laboratories which already enjoyed a world wide reputation. Doctor Chalmers maintained this reputation and extended it. He had resigned his post as Director and was in India on his way home when stricken down.

Doctor Chalmer's own work was of the highest type, abundant in quality and characterized by both accuracy and thoroughness. It was also marked by the generous appreciation accorded the work of colleagues and the full measure of credit given to associates in the laboratory. In addition to numerous separate publications in parasitology, especially on mycetoma, Doctor Chalmers is most widely known as author of the splendid Manual of Tropical Medicine written in cooperation with Doctor Aldo Castellani.

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The actual dates of issue of Volume VI of The Journal were as follows:

No. 1, October 29, 1919 — No. 3, May 19, 1920
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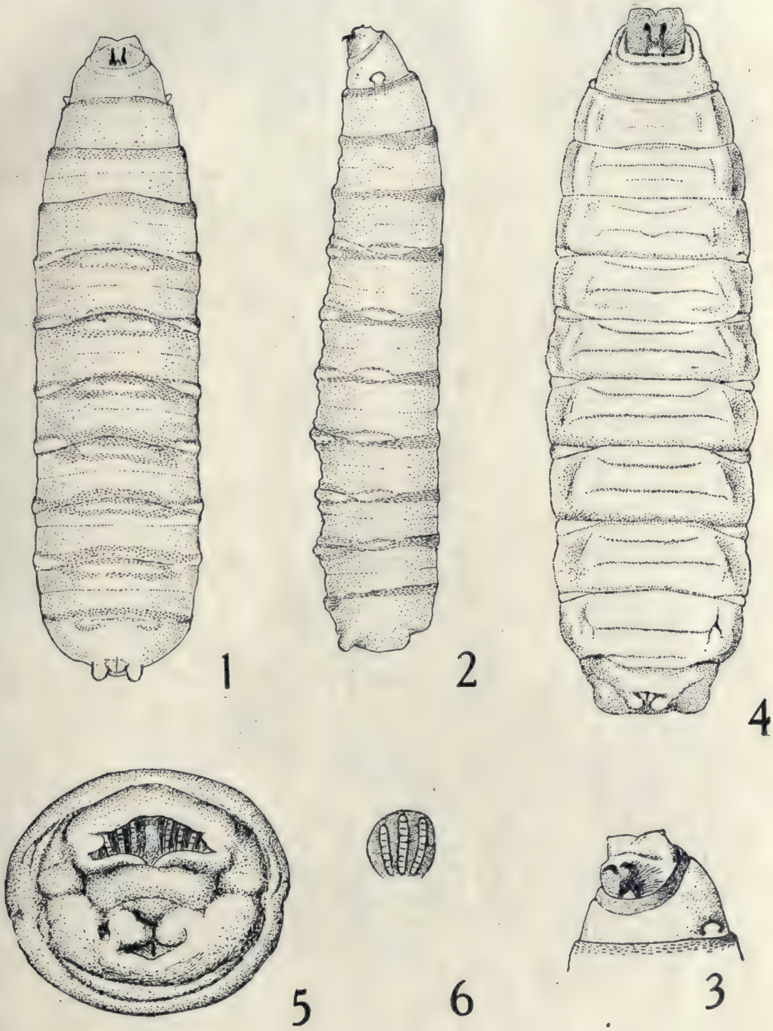

PLATE I

Fig. 1.—*Wohlfahrtia vigil* (Walk.). Larva from first case, ventral view.

Fig. 2.—Same larva, lateral view.

Fig. 3.—Same larva, ventrolateral view of anterior segments.

Fig. 4.—*Wohlfahrtia vigil* (Walk.). Larva from second case, ventral view.

Fig. 5.—Same larva, posterior view.

Fig. 6.—Same larva, left posterior spiracle.

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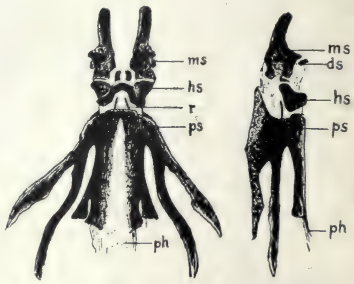

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PLATE II

Fig. 7.—*Wohlfahrtia vigil* (Walk.). Ventral view of puparium.

Fig. 8.—*Wohlfahrtia vigil* (Walk.). Cephalo-pharyngeal sclerites, ventral view; *ms,* mandibular sclerite; *ds,* dental sclerite; *hs,* hypostomal sclerite; *ps,* pharyngeal sclerite; *r,* rod-like processes of pharyngeal sclerite; *ph,* floor or pharynx.

Fig. 9.—Same, lateral view, lettering as before.

Fig. 10.—*Wohlfahrtia vigil* (Walk.). Adult male.

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WOHLFAHRTIA VIGIL (WALKER) AS A HUMAN PARASITE (DIPTERA—SARCOPHAGIDAE)

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On June 15, 1919, a Toronto surgeon called the writer by telephone and described a case of boil-like sores in an infant, from some of which he had removed small, whitish maggots. On my request to see the latter, the child and its mother were brought by the surgeon to my house, where the larvae were removed.

The patient was a female infant, two weeks old, in fair condition, though irritable almost to the point of exhaustion. The sores consisted of twelve somewhat swollen, inflamed areas, about one to two and a half centimeters in diameter, scattered over the front of the neck and arms, palms and chest. One of the palms was particularly red and swollen. On each sore there was a minute and very inconspicuous external opening. From these sores nine dipterous maggots were extracted, each from a different sore, although as some of the sores had been previously opened, it is possible that one or two of them may have contained more than a single larva. Another was removed from a spot on the shoulder on the following day, according to the mother's statement, after which operation the child recovered rapidly.

The larvae, all but two of which were more or less injured in the operation, were preserved in alcohol. They vary in length from 2.5 mm. to 4 mm., but this variation is partly due to the contraction of the injured specimens and the considerable extension of the largest uninjured one. All appear to belong to the same stage, though there is some difference in the individual size of the head segment and mouth-parts.

The sores had been noticed by the mother for the first time on the evening of June 13. The mother states that the baby had not been sleeping out of doors on that day but that the front door had been left open, so that the fly must have entered the house to deposit its young. The house is in a fairly densely populated section of North Toronto, but is less than half a mile from the ravine and wooded country in the vicinity of Reservoir Park.

A second case, very similar to the above, was brought to my notice on June 23 by a Toronto physician. The child, whose home was in West Toronto, was admitted to the Sick Children's Hospital on this date, and I visited the hospital in the afternoon. The patient was a female infant, eight weeks old, well grown and well nourished. There were fourteen lesions distributed upon the front of the neck, chest and anterior surface of the arms. They were of somewhat larger size than those of the first case, averaging about 2 cm. in diameter. Those on the neck were particularly swollen and inflamed. The mother had not observed anything wrong with the baby until three days previous to its admission to the hospital, when pimples appeared on the neck. On the twenty-second "worms were seen to come from the pimples."

Each swelling had a round or elliptical opening about 3 mm. in diameter and from some of these larvae were squeezed out. Usually there was a single larva in each swelling but from one of them three larvae were expelled.

When I visited the hospital some eight or nine larvae had been removed and placed upon raw beef in an incubator at body temperature. The surgeon was just completing the extraction of the remainder (about ten), which were kindly given to me for investigation. I placed them upon some raw beef in a test tube and took them home. According to the hospital record these larvae varied in length from about 5 to 15 mm. The latter measurement appears to me excessive unless the specimens were fully extended.

On the following day (June 24) the larvae had greatly increased in size and were very active, feeding on the underside of the meat. One, however, had crawled a little way up the side of the tube and, being unable to return, had died. This was preserved in 70% alcohol. On the next day the larvae were apparently full grown, measuring about 17 or 18 mm. in length (exact measurements were not made). The meat upon which they had been feeding had become extremely putrid, so I introduced a fresh piece, but they did not touch it.

On the morning of June 26 I removed the larvae and meat to a half pint jar, which I partly filled with slightly moist earth to a depth of about four inches. I then observed that only four larvae were present, all of them evidently full grown. No trace of the others remained. They could not have escaped from the tube, which was kept upright and plugged with cotton wool, and it would therefore appear that they had been devoured by the survivors. This was unfortunate, as I had intended to preserve one or two of the full grown larvae.

On the same day I took the jar containing the larvae to DeGrassi Point, Lake Simcoe, where on the following morning they had commenced to burrow into the earth. On June 28 two of the larvae were still at the surface but were becoming shorter and more oval in form. On the 29th all were beneath the surface, but two could be seen through the side of the jar and were moving somewhat actively. After this they disappeared from view.

On July 4 I dug them up. All had transformed to puparia and were at or near the bottom of the jar. I placed them upon somewhat drier earth in a breeding cage.

On July 18, about 9 p. m., I looked into the cage and saw four soft-looking pale gray flies with wings not yet expanded, crawling about the sides of the cage. One of them, while walking over the mosquito netting which covered the front of the cage, thrust its ptilinum through one of the meshes and had to be pushed back. The wings were not fully expanded until 11 a. m.

I then smeared some sweetened milk on the netting, which they devoured greedily. By the afternoon they were very active, running up and down the walls of the cage. They were readily recognized as Sarcophagids but had, to me, an unfamiliar appearance. I kept them until July 24, feeding them upon sweetened diluted milk and jam, which they took at all times of the day very readily, but on this date I found that two had died and the others were somewhat sluggish, so that, relinquishing the hope of obtaining fertilized eggs I killed the remaining two. Up to this date they had been very active.

The larvae that were kept at the hospital were reported to have "grown to two or three times their former size" on June 26, but were unfortunately destroyed when the meat upon which they were feeding became very putrid.

As in the first case the child recovered rapidly after the removal of the larvae, no secondary infections having developed.

The four flies obtained consisted of one male and three females and were readily determined from Mr. Aldrich's admirable monograph as *Wohlfahrtia vigil* (Walker) and Mr. Aldrich, to whom I sent one of the specimens, kindly confirmed my determination.

The genus Wohlfahrtia was erected by Aldrich (1916) for certain Sarcophagid flies formerly included in the genus Sarcophila and the type species *W. magnifica* (Schiner) (*Sarcophila wohlfahrti* Portchinsky) has long been known as a parasite of man and various domestic animals, particularly in Russia, having been regarded as the European analogue of the Screw-worm Fly (*Compsomyia macellaria* Fabr.) of the warmer parts of America. Such habits are unknown, however, for the other European species of Wohlfahrtia, one of which, *W.*

meigenii, occurs also in western North America and is so closely related to the eastern species, *W. vigil,* as to be perhaps only a race of this species (vide Aldrich, 1916).

Nothing has been hitherto known of the larval habits of the North American species of Wohlfahrtia. Concerning *W. magnifica* several valuable papers were published between the years 1874 and 1876 by Joseph Portchinsky of St. Petersburg. A review of this work by Osten Sacken appeared in 1877 and a copy of this was very kindly sent to me by Mr. Aldrich. In this review is the following paragraph:

"In 1875-76 Portchinski published an elaborate paper, entitled 'Materials for the natural history of the flies which, in their larval stage, cause diseases among men and animals' (Trudy, etc., vol. ix, p. 3-180, with three plates). A condensation of a portion of this paper concerning *Sarcophila wohlfahrti* was published in the Horae Soc. Ent. Ross., vol. xi, 1875, pp. 123-180, in German under the title 'Krankheiten welche im Mohilewschen Gouvernment von Larven von *Sarcophila wohlfahrtia* entstehen und deren Biologie.' In 1884 a monographic essay on *Sarcophila wohlfahrti* appeared (Horae, etc., vol. xviii, p. 247-314, with 33 woodcuts), containing some new observations and comparative descriptions of this fly and its next relatives."

On Mr. Aldrich's suggestion and through the kindness of the Secretary of the Smithsonian Institution I obtained the loan of a copy of the former volume, in which Portchinsky describes a number of cases of human myiasis, caused by *Wohlfahrtia magnifica* (*Sarcophila wohlfahrti*), chiefly in children under 13 years of age. In all these cases the larvae are described as feeding gregariously upon the mucous membrane and underlying tissues of the ear, nose, gums or even the eye, or in one case an eczematous scalp; but no mention is made of the larvae ever penetrating the healthy skin, as must have occurred in the two cases of infection by *W. vigil.* In the case of a five year old boy who had a copious discharge of blood and pus from the nose there were six round openings on the upper lip, close to the nostrils, but these were probably not points of entrance as they appeared to communicate with the frontal sinus by way of the nasal cavity. The larvae would frequently come to the surface through these passages and would sometimes protrude considerably from the openings. In the cases of infection by *W. vigil* the scattered distribution of the lesions, as well as the penetration of the skin by the larvae, seem to indicate a distinctive habit, but the apparent difference from *W. magnifica* in this respect may be due merely to the difference in the ages of the hosts; the healthy skin, except that of very young infants, being perhaps impenetrable by the young larvae of either species.

In this connection it may be worth while to record that a farmer residing near Port Sydney, Ont., who is also a keen naturalist, told me that a few years ago he had suffered from severe pains in the nose, accompanied by a sensation as though something were creeping within it, and that, after a violent sneezing fit, a large maggot had dropped out; after which the trouble subsided. The capture of a specimen of *Wohlfahrtia vigil* in this locality, by Mr. N. K. Bigelow, indicates the possibility of the larva having belonged to this species.

While it is impossible to prove that the larvae from the first case belong to the same species as those from the second, the clinical features of the two cases were so very similar that I have no hesitation in considering them both to belong to *Wohlfahrtia vigil,* in spite of certain differences which are described below. These differences are not surprising in larvae which represent different stages of development; they are in fact less than those which occur in *W. magnifica.*

Figures 1 and 2 represent the largest of the larvae taken from the first case. It measures 4 mm. in length but is fully extended and agrees in all other respects with the smaller larvae from the same case. Figure 3 is a ventro-lateral view of the head of the same larva.

The two lobes into which the upper part of the pseudocephalon (cephalic segment) is divided appear somewhat less prominent than in the 3 lines long larva of *W. magnifica* figured by Portchinsky (see Osten-Sacken, 11. pl. 4, fig. 1) and the mandibular sclerites (lateral hooks) are shorter and blunter. The anterior spiracular processes are much broader than long and bear 9 to 10 minute spiracular papillae, whereas that of *W. magnifica,* figured from a 2½ lines long larva, is much longer than broad and terminates in only 4 papillae, which are long and capitate. The posterior spiracles in *W. vigil* at this stage, which is probably the second, have only two openings.

The spinules of the trunk segments are very much smaller and more restricted in distribution than in *W. magnifica.* Those of the second segment (strictly the united second and third) form a narrow ring at the front margin and are so minute as to be invisible except under high magnification (Fig. 3). Those of the other segments are larger and visible under much lower powers, but are nevertheless very minute. They are arranged, for the most part, in small, subtransverse groups of two to four. Those of the third, fourth and fifth segments are arranged in a single ring at the anterior margin of each segment, that of the third segment very narrow, those of the fourth and fifth increasing in width to nearly one third the width of the latter segment. On the remaining segments the rings of spinules are similar on the dorsal surface, but more irregular ventrally and laterally. On the ventral surface they form a transverse patch, occupying the

anterior third or more of the length of the segment and enclosing a transversely elongate bare area. These patches are confluent with a narrow band along the caudal margin of the next preceding segment, and the latter bands are continuous or subcontinuous laterally with a narrow strip of spinules along the front of the lateral fold.

In the smallest larva of *W. magnifica* figured by Portchinsky the spinules are much larger and not arranged in small groups. The spinulose bands are much more extensive. That of the second segment is much wider though described as narrow, and consists of very small spinules. The third segment is described as being bare in the middle, but provided with spinules on the front and hind margins; the third segment is entirely covered with spinules below, except a narrow bare band on the hind margin and a likewise bare, triangular, elongate space, which lies about the middle of the segment. Segments 4 to 6 are similar to segments 5 to 7 in *W. vigil* except that the spinulose bands are much broader and the enclosed bare area is divided transversely by a row of spinules. The two following segments are wholly covered with spinules, except for a narrow bare area on each and the lateral folds. Segment 9 is armed with spinules only on the anterior half, with only a few rows of spinules on the posterior half. Segment 10 presents almost the same pattern, except that the spinulose band on the front margin is narrower and the remainder of the segment almost naked. The last segment is almost entirely naked, being provided with spinules only on the middle of the front margin and at the base of the two anal papillae.

The single larva of *W. vigil* that was preserved from the second case (Figs. 4-6) belongs to a later stage than those from the first case, and though only 7 mm. long, probably shows the characteristics of the mature larva. The pseudocephalon is similar to that of the larva described above, except that the outline as seen from below is nearly square, the sides being parallel and the emargination of the front narrower. The mandibular sclerites are somewhat blunter and less curved. The posterior spiracles have three slits, like those of *W. magnifica* and other Sarcophagids. The anterior spiracular processes are like those of the earlier stage, being very short and broad, with an arcuate margin bearing 9 papillae. The spinules are wholly absent, being represented only by minute granulations which are invisible except under high magnification. They are difficult to see in the single larva preserved, but can be readily distinguished in the puparia, in which their arrangement is seen to be essentially the same as that of the spinules in the young larva (*cf.* Figs. 1 and 7).

It is worthy of note that whereas in this species the spinules are lost during development, in *W. magnifica* according to Portchinsky, they increase in both number and size, as shown in two successive

instars described and figured on plate III of the work cited above.. Portchinsky believes that the great development of spines in this species is connected with its parasitic life. If this be true it would appear probable that the parasitic habit is abnormal in *W. vigil,* in which the cuticle is even less spiny than in many muscoid larvae that develop in dead organic matter.

Puparium. (Fig. 7). Length 9 to 10 mm., diameter slightly less than half the length; the ends slightly flattened; segments marked with narrow, slightly roughened bands having essentially the same arrangement as the spinules of the young larva (Fig. 1); pocket enclosing the posterior spiracles with a slightly raised margin.

In order to examine the cephalo-pharyngeal sclerites, these were removed from the inner surface of one of the empty puparia, and are shown in Figs. 8 and 9. The pharyngeal sclerites (*ps*)* are united in front, both dorsally and ventrally. Each is prolonged behind into three processes, a ventral, continuous with the floor of the pharynx, widening somewhat caudad, with a narrow, thinly chitinized area next to the hind margin, a dorsal, slightly longer, directed ectocaudad and somewhat expanded distally, and a lateral, considerably longer than either of the others, somewhat sinuate, with slender apices. The notch between the ventral and lateral processes is somewhat deeper than that between the middle and dorsal processes. The hypostomal sclerite (*hs*) consists of two subtriangular, ventrolateral plates, united by a ventral arch with the concavity caudad. Behind and above the arch is a pair of slender, rod-like processes of the pharyngeal sclerite (*r*), and in front of the arch is a pair of small, short sclerites. The mandibular sclerites (*ms*) are not much elongated, and are rather blunt and but little decurved. Their proximal half is stout and bears a prominent ventral tooth, with which is connected the small dental sclerite (*ds*).

The female adults measure 10.5 to 11 mm. in length, the male 13 mm. The male from Port Sydney, Ont., is 12 mm. long.

References Cited

Aldrich, J. M. 1916.—Sarcophaga and its Allies in North America. Thos. Say Foundation, pp. 25-30.

Hewitt, C. G. 1914.—The House-fly, Musca domestica Linn. Cambridge Zool. Series, 382 pp.

Osten Sacken, C. R. 1887.—On Mr. Portchinski's publications on the larvae of Muscidae including a detailed abstract of his last paper; Comparative biology of the necrophagous and coprophagous larvae. Berliner Ent. Zeitsch., 31, no. 1.

* The terminology followed here is that of C. G. Hewitt (1914:134).

A NEW CYSTOPHOROUS CERCARIA FROM CALIFORNIA *

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A number of specimens of *Physa occidentalis* collected from Lake Temescal, a city reservoir near Oakland, California, were heavily infested with a new cercaria, to which the name *Cercaria californiensis* spec. nov. will be given. The infection was localized in the digestive gland of the snail. At the time that the examinations were made (September to December) there were found in the infected snails only rediae which contained cercariae. No sporocysts or rediae containing rediae were found.

The rediae of this species vary considerably in size, ranging from 0.5 mm. to 1.6 mm. in length and have a width equal to about one-fifth or one-fourth the length. They are elongate sacs, slightly narrowed at the anterior end, and are without lateral projections. The body walls of a few of these rediae contain a pale orange pigment, the others being quite transparent. Contrary to what is usually observed in pigmented parthenitae, the pigment in this species appears in the immature rediae, the older ones being always unpigmented. The pharynx is small and the intestine extends about one-fourth of the total length. No birth pore is present. When the rediae are first freed from the digestive gland of the snail host they contract and expand actively for a few minutes, but are unable to move from place to place. Cercariae and developing germ balls fill the body cavity of the rediae from the posterior end to the region just behind the pharynx. The number of fully developed cercariae contained within a redia varies from eight to fifty. This great variation in number can easily be explained since in the absence of a birth pore all the cercariae which develop must remain within the redia.

The body of *Cercaria californiensis* (Fig. 2) is very small, having a length varying from 0.12 mm. to 0.18 mm. according to the state of contraction. The attachment between the tail and the body is weak, and is usually broken soon after the cercaria is freed

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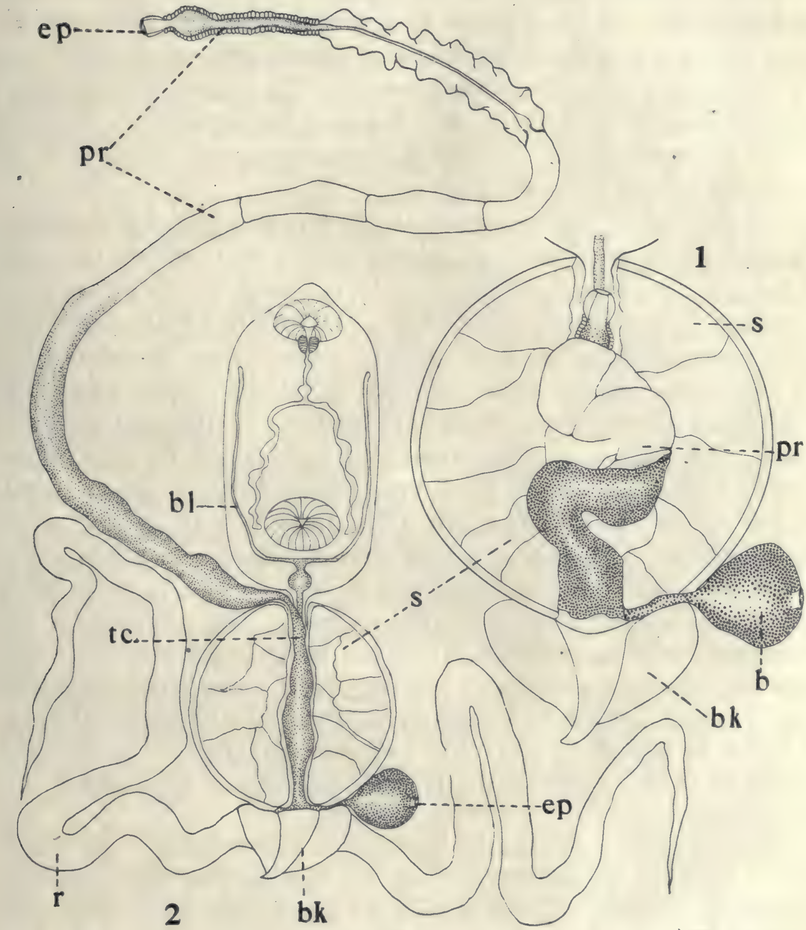
from the redia for study. While still attached to the tail the cercaria was never seen to move about, but when it had broken loose it could locomote slowly by the use of its suckers. The body cannot be contracted into the tail, but always keeps the relation to the tail shown in figure 1 while within the redia and, when freed from the



redia, the position shown in figure 2. The size and position of the various organs of the body can be made out readily from figure 2. The ventral sucker is slightly larger than the oral and is in the posterior third of the body.

The mouth of *C. californiensis* is on the ventral side a little distance back of the pointed anterior tip. There is no prepharynx.

The pharynx is small and is followed by an esophagus of medium length (Fig. 2). The intestinal ceca extend back to the level of the ventral sucker.

Of the excretory system only the parts of the bladder were distinguished (Fig. 2, *bl*). The undivided portion of the bladder has a globular enlargement containing the highly refractive concretions which are so comonly found in the excretory bladder of cercariae and agamodistomes. It is connected by a narrow tube with the excretory tubes of the tail. The bifurcations of the bladder were traced forward to a point just back of the pharynx. The region of the bifurcations nearest the enlargement and the tube leading into the tail also contain the concretions. The peculiar relations of the excretory canals in the tail will be discussed later.

The tail of *C. californiensis* is very remarkably differentiated. Figure 1 shows its appearance when the cercaria is within the redia or just after it has been forced out. In this condition the tail appears as a large firm cuticular sphere (Fig. 1, *s*) with two short projections at its posterior end (Fig. 1, *bk* and *b*). One of these projections has the form of a cuticular beak (*bk*). It is entirely transparent and is divided into a central area which is pointed posteriorly and two lateral areas. The beak is so transparent that nothing can be seen of the ribbons (Fig. 2, *r*) which are later extruded from the lateral areas. To one side of the beak is a bulb (Fig. 1, *b*) with an opening on its outer surface. This bulb is loaded with excretory concretions. The sphere has a thick cuticular wall and is very difficult to break when pressure is applied on the cover glass above it. The sphere has a central column of living material (Fig. 2, *tc*) along which is folded a very complex cylindrical structure. The portion of the sphere between the central column and the wall is highly vacuolated. The posterior region of this body is directly connected with the column of the sphere, and a tube from the excretory bladder of the body extends down into this column.

Soon after the cercaria is freed from the redia two flat transparent cuticular ribbons (Fig. 2, *r*) are extended out laterally from the sides of the beak. These ribbons are pointed and have no apparent structural differentiation. At the same time there is slowly protruded from the place where the body joins the tail sphere the long cylindrical projection which, in the condition indicated in figure 1, is folded along the column of the sphere (Fig. 1, *pr*). This structure, which will be called the excretory projection, is protruded until it has a length more than three times that of the body of the cercaria (Fig. 2, *pr*). The excretory projection is a hollow cuticular cylinder. The proximal half of this cylinder is undifferentiated, has a very

thin wall and contains excretory concretions. Beyond the middle the excretory projection appears to be jointed externally. At about the beginning of the distal third of the excretory projection the cuticular wall becomes much thicker, narrowing the lumen. In the first half of this region the greatly thickened cuticula appears to be ruffled. Distal to this region the lumen widens slightly and the cuticula appears to be fluted. The excretory projection ends with a dilation followed by a cup-like structure with a large opening at its end. The first half of the excretory projection and the last fluted part contain excretory concretions. The excretory projection is broadly attached to the posterior part of the column of the tail (Fig. 2, *tc*). The differentiations of the excretory projection are shown in figure 2.

The presence of excretory concretions in various parts of the tail of *C. californiensis* indicates the relation of these parts to the excretory system. There are two openings of the excretory system in the tail (Fig. 2, *ep*), one on the outer surface of the excretory bulb, and the other at the end of the excretory projection. The excretory bulb is directly continuous with a tube which runs from the bladder of the body down the column of the tail. The lumen of the excretory projection is also directly connected with this tube. These connections are indicated by the presence of the concretions in the various structures. Movements of the concretions could be followed from the tube in the column of the tail into the excretory bulb. Excreta from the body of *C. californiensis* can escape after passing down the column of the tail-sphere either by the pore of the excretory bulb or after passing along the excretory projection from the opening at its end.

Apart from the relation to the excretory system described above, it is difficult to suggest functions for the various parts of the tail of *C. californiensis*. The function of the tail of a cercaria is evidently to aid it in that period of activity, usually including a short period of free life, from the time it leaves its parthenita until it is settled in its secondary intermediate host, its final host, or is encysted in the open waiting to be taken into its final host. We have no direct evidence in regard to the further development or activities of *C. californiensis*. There are certain things, however, which it is perfectly evident that this cercaria does not do. The absence of a stylet and cephalic glands and the weak muscular development of the body of the cercaria make it evident that it does not penetrate actively into the next host. The absence of cystogenous glands in the body and the inability to withdraw into the vesicle of the tail show that it does not encyst in the open and wait to be taken into its next host. Finally

the fact that the redia has no birth pore, that the cercariae accumulate in the redia and that they are practically incapable of locomotion in the open, makes it very probable that this cercaria never leaves its intermediate host and continues its development in some vertebrate which feeds on this host.

There is nothing in such a life cycle which would make it possible to explain the complex structure of the tail of *C. californiensis* in terms of function. The fact that in three closely related forms *C. cystophora* (Wagener, 1866), *C. sagittarius* (Ssinitzin, 1911: 15-19), and *C. yoshidae** (Yoshida, 1917) the body of the cercaria can be withdrawn into the tail for protection suggests that this is the primary function of such a vesicle as the sphere of *C. californiensis.* A change in life cycle which eliminated the free stage and therefore the importance of this function may have led to the loss of the power of withdrawal into the tail vesicle without the loss of that structure. It is still more difficult to determine any function for the appendages of the tail. Ssinitsin, (1911: 18) suggests that the various appendages of the tail of *C. sagittarius* hold the tail vesicle, which forms a cyst containing the cercaria, in position in the digestive tract of the final host until the cercaria has had a chance to get out of its cyst. Such a function for the excretory projection and ribbons of *C. californiensis* seems very improbable since in the first place this cercaria does not become encysted in the vesicle of the tail and in the second place the body is so weakly attached to the tail that even a slight pull will break the connection. It is, of course, possible that these appendages may also represent structures which have lost their function through modifications of the life cycle. Finally it is very probable that structures may develop in connection with the evolution of such a diversified organ as the cercaria tail which have no function whatsoever.

C. californiensis belongs to a small group of cercariae which Ssinitsin (1911: 20) named the Cystophrous Cercariae. He characterizes this group as containing cercaria which possess a vesicular tail with various appendages. The first cercaria of this type to be described was *Cercaria cystophora* which Wagener (1866) reported from *Planorbis marginatus.* Another cercaria which probably belongs to this group was described by Sonsino (1892) from *Cleopatra bulimoides* as *C. capsularis.* Sonsino's original description is hardly sufficiently detailed to make the relationship of this species clear. The same cercaria is later described in the immature state by Looss (1896). These two descriptions taken together make it pretty certain that

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* I use this name to designate the species which Yoshida describes as Cercaria F.

this form belongs to the cystophorous cercariae, but do not give enough data to make a detailed comparison possible. Pelseneer (1906) described two marine representatives of this group, *C. appendiculata* from *Natica alderi* and *C. vaullegeardi* from *Trochus cinerarius.* Later Ssinitzin (1911: 15-21) described two more marine cystophorous cercariae, *C. sagittarius* from *Cerithialum exille* and *C. laqueator* from *Rissoa venusta.* Finally there should be added to this group *C. yoshidae* (Yoshida, 1917) from *Melania libertina* from Japan and *C. californiensis.* While the cystophorous cercariae vary considerably in structural details, they have so many important points in common, that we consider that they form a natural group.

All the cystophorous cercariae except *C. vaullegeardi* (Pelseneer) develop in rediae. Mother sporocysts in which the rediae develop have been described for two species, *C. sagittarius* Ssinitzin (1911: 15) and *C. cystophora* (Wagener, 1866). In none of the species were rediae which contained rediae found and the descriptions of the two species of which the mother sporocysts were found, indicates that such a stage is not present in the life histories of the members of this group. The rediae are much alike having no locomotor appendages, a very small pharynx and a voluminous digestive tract varying from one-fourth the length of the redia in *C. californiensis* to two-thirds its length in *C. yoshidae.*

The body of the cercariae in all cystophorous cercariae is very small and the primordial adult characters are very slightly developed. This is indicated by the small size, the lack of clear differentiation of the organs and the short distance between the ventral sucker and the posterior end. The bodies of these cercariae lack entirely all adaptive larval structures for penetration and encystment, i. e., stylet, cephalic glands and cystogenous glands. The digestive system in those forms for which it has been described is much like that of *C. californiensis* (Fig. 1), except that the length of the intestinal ceca varies. The excretory bladder is y-shaped.

The tails in these forms differ considerably in detail, but are all built on the same fundamental plan. There is present in each species a central vesicle or sphere as we have called it in *C. californiensis.* This is a firm structure with a thick cuticular wall which was evidently developed to function as a cyst into which the cercaria can be withdrawn into the cavity of the sphere as in *C. sagittarius, C. cystophora, C. yoshidae.* In regard to *C. laqueator* and *C. californiensis* the statement is made that the body of the cercaria cannot be withdrawn into the vesicle of the tail. It is evident that this ability of the cercaria to withdraw into the tail would be a very important protective measure

at the time of entrance of the cercaria into its next host. Besides the central vesicle there is found in every species of this group a protractile appendage comparable to the structure we have called the excretory projection in *C. californiensis*. This structure differs greatly in the various species and in some is not sufficiently described for comparison. The various other appendages which are found on the tails of the Cystophorous cercariae are so remarkably varied and grotesque that it is not only impossible to get an idea of what their function may be, but also there seems to be no homologies which can be worked out between them.

Further development is known for only one of the cystophorous cercariae, *C. cystophora*, which develops into *Halipegus ovicaudatus* which is found in the mouth cavity of the European frog. Ssinitzin (1905 and 1907) finds the agamodistine stage of this species free in the body cavity of the nymph of the dragon-fly *Calopteryx virgo*. On this account he suggests (Ssinitzin, 1911:21) that the life cycles of the other members of this group will probably follow the same course. This deduction does not seem to us to necessarily follow since a fundamental modification of life cycle might come to a species, and yet the structural adaptations for the previous mode of life might be retained. Certainly in the case of *C. californiensis* there could hardly be a secondary intermediate host, since it seems very improbable that this cercaria ever leaves its molluscan intermediary.

SUMMARY

1. *Cercaria californiensis*, a new species of cercaria from *Physa occidentalis*, has a very small body which shows no adaptive larval characters and but slight development of primordial adult characters.

2. The tail of this species is very remarkably differentiated, consisting of a central cuticular vesicle, the sphere, and various projections and appendages.

3. It is not possible to explain the functions of the parts of this tail in terms of activities of the cercaria since the cercaria probably never leaves the redia within its intermediate host.

4. *Cercaria californiensis* belongs to a natural group, the cystophorous cercariae which are characterized by a small, very slightly differentiated body and an extremely complex tail consisting of a central cuticular vesicle, with various appendages.

Literature Cited

Looss, A. 1896.—Recherches sur la faune parasitaire de l' Egypte. Premiére partie. Mem. l'institute Egyptien, 3:1-252; 16 pls.

Pelseneer, P. 1906.—Trematodes parasites de mollusques marins. Bull. scien. France et Belg., 40:161-186; 5 pls.

Sonsino, P. 1892.—Studi sui parassiti molluschi di acqua dolce dintorni di Cairo in Egitto. Leuckart's Festschr., pp. 134-147.

Ssinitzin, D. Th. 1905.—Studies on the life-cycle of trematodes. The Distomes of the Fish and Frogs in the Environs of Warsaw. (Russian.) Mem. soc. nat. Varsovie biol., 15:1-210, 6 pls.

1907.—Observations zur les métamorphoses des Trématodes. Arch. zool. expér et géner., 37, notes et rev., pp. 21-27.

1911.—The Parthenogenetic Generation of Trematodes and their Progeny in the Molluscs of the Black Sea. (Russian) Records Imp. Acad. Sci., (8) 30:1-127; 6 pls.

Wagener, G. R. 1866.—Ueber Redien und Sporocysten Filippi. Arch. Anat., Physiol., u. wissensch. Med., 145-150. 6 pls.

Yoshida, S. 1917.—On the Cercariae from Melania in Japan. (Japanese) Dobutsu Jaku Zasshi (Zoological Magazine) vol. 29, no. 342.

ON THE NATURAL OCCURRENCE OF HERPETOMONADS (LEPTOMONADS) IN THE BLOOD OF A FISH, *DENTEX ARGYROZONA*, AND ITS SIGNIFICANCE

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During the years 1908-1916, we were conducting researches, individually and in collaboration, on the life-cycles of Trypanosomes, Crithidia and Herpetomonas and their significance in the evolution of disease. After working on the life-histories of the Protozoa used by us, we were able to show, by direct experiment, that Herpetomonads and Crithidia could be inoculated into or fed to vertebrates and produce therein pathogenic effects resembling those of leishmaniasis. We also found natural occurrences of herpetomonads in mice, and in 1916 summarized our experimental conclusions to date, when war-work on the diagnosis of protozoal diseases completely stopped our further progress. Recently (Jan.-Feb., 1919), while working at the St. James Marine Aquarium, near Cape Town, we were agreeably surprised to find a Herpetomonas in the heart blood of a freshly killed silver-fish, *Dentex argyrozona.* Subsequently, the same organism was found in small numbers in the blood and organs of three more Dentex, a total of 4 out of 41 examined containing the Herpetomonas, but in each case the infection was scanty.

As no mention of such a parasite in the blood of fish can be found in the literature available, we propose to describe the Herpetomonas, and to name it *Herpetomonas denticis.* It is true, that, morphologically, it is somewhat difficult to separate this species from others, but the occurrence of physiological species or races is known, and such may be the case here. New and unexpected methods of research in future may shed further light on physiological species; at present we think that we are justified in giving a separate name to the Herpetomonas of *Dentex argyrozona* for purposes of reference. We regret that, as a result of our comparative studies, we are unable to accept a biflagellate character as diagnostic of the genus Herpetomonas, and it is also unfortunate that *Leptomonas gracilis,* the type species of Leptomonas, has not been studied by modern methods. We therefore accept Butschli's (1884) definition of the genus Herpetomonas.

As indicated in the introduction, the herpetomonads were found in the blood and organs of freshly killed silver-fish, *Dentex argyrozona*. Fresh preparations of the blood taken direct from the heart were examined both directly and by the aid of the paraboloid condenser, and stained preparations of both heart blood and internal organs of the fish were examined. Fixation by exposure to osmic acid vapor and formalin vapor followed by absolute alcohol, and direct wet fixation with Schaudinn, Carnoy or Bouin-Duboscq fluids were tried, while Giemsa, Delafield's hematoxylin and iron-hematoxylin stains were used. The most useful preparation was one fixed with osmic vapor and absolute alcohol and stained with Giemsa's solution.

DISTRIBUTION OF THE PARASITE IN THE HOST

At no time was *Herpetomonas denticis* abundant in any fish that we examined. It was seen very rarely and with difficulty in life. The parasites were most often found in stained smears made from the heart blood of the host. A few were found in the spleen and liver. Other organs were rarely infected, though a few parasites have been observed in a preparation from the kidney of one host. Intracellular forms have not been observed.

The herpetomonads were present both in the flagellate and the non-flagellate phases. As a general statement, flagellate forms were more common in the heart blood and non-flagellate forms in preparations of organs such as the spleen.

No herpetomonads were seen in the gut of any of the Dentex, though careful search was made. No marked pathologic effects on the hosts were observed.

MORPHOLOGY

The flagellate form of *Herpetomonas denticis* is small, the body measuring from 5 to 24μ long and 1.5 to 2.5μ broad (see photomicrographs). The variation in length is rather great, the short-bodied forms being apparently younger. The flagellum is often longer than the body, especially in young flagellate forms, as, for example, in a parasite whose body length was 7.5μ and length of free flagellum was 16μ. The posterior (non-flagellate) end of the body was sometimes pointed, at other times rounded.

The general cytoplasm was almost homogeneous, though in some specimens a finely alveolar structure was seen. Chromated granules may be seen in some specimens.

The nucleus was karyosomatic in some cases, and finely granular in others, the structure varying as we have pointed out previously, with the degree of activity of the cell-life. Prior to periods of great

multiplicative activity, the nucleus usually becomes finely granular in a flagellate, and such changes can be observed in the living organism under favorable conditions.

The blepharoplast, or kinetic nucleus of some authors, is distinct and often bar-like, but sometimes is slightly curved or rounded, the latter appearance probably being due to an end-on view. The organella may be surrounded by a less deeply staining area of cytoplasm.

The flagellum arises in the neighborhood of the blepharoplast but not from it. The root of the single flagellum is usually well marked.

The non-flagellate stages are small, oval or somewhat pyriform bodies, possessing a nucleus and distinct blepharoplast. The small oval or leishmaniform parasites measure 2.5 to 4.5μ by 1.5 to 2.5μ. Larger forms, elongating into flagellates though still lacking a flagellum, may be somewhat longer and broader.

Multiplication by fission occurs among both flagellate and non-flagellate forms. Division begins in the blepharoplast and is followed by division of the nucleus. Longitudinal fission was seen in flagellate parasites and in several intermediate elongating forms.

The occurrence of division shows that the herpetomonad could increase in numbers in the Dentex, and so was more than a mere conservation of the organism.

SIGNIFICANCE OF NATURAL HERPETOMONADS IN VERTEBRATES

The significance of the findings of herpetomonads in the blood of representatives of most classes of vertebrates is most important. The published results of our personal work on the life-histories of Herpetomonads and Crithidia have been strongly indicative that leishmaniases—such as kala-azar, dermo-mucosal and dermal leishmaniases—were really due to herpetomonads being able to live in the blood of vertebrates. Further, we have conducted experiments on the inoculation and the feeding of herpetomonads and a few crithidia to all classes of vertebrates with positive results. Laveran and Franchini have performed similar experiments and Laveran has shown that Leishmania can be inoculated into cold-blooded vertebrates. These various experiments have been carefully discussed recently by Laveran in his monograph on "Leishmanioses." We have had the good fortune to find herpetomonads occurring naturally in the blood of mice and of Dentex.

At present, herpetomonads have been found occurring naturally in the blood of the following vertebrates.

(1) Man. In 1913 a herpetomonad was described by Franchini from the blood and internal organs of man. Unfortunately, the name *Haemocystozoon brasiliense* was given to the organism. An allied parasite was recently found by M. Léger in French Guiana, herpeto-

monad and trypanosome forms being seen. It should also be mentioned here that herpetomonad flagellate forms of Leishmania have been seen in man.

(2) Mice. Herpetomonads were seen in the blood of Gambian mice by Dutton and Todd in 1903, while Fantham and Porter published similar observations on the natural occurrence of herpetomonads in the blood of English mice in 1915, these having been seen from time to time during the previous six years.

(3) Pigeons. Natural infections of these birds by a herpetomonad was found by Edmond and Etienne Sergent in 1907 in Algeria.

(4) Reptiles. Natural infection of geckos in Algeria with herpetomonads in the blood was found in 1914 by Sergent, Lemaire and Senevet. The Herpetomonads of geckos was also found by Chatton and Blanc in Tunis in 1918. Marcel Léger in 1918 found herpetomonads in the blood of small lizards belonging to the genus Anolis in Martinique.

(5) Fishes. A herpetomonas occurring in the blood and internal organs of *Dentex argyrozona* is now recorded by us.

As before mentioned, we were able to produce herpetomoniasis experimentally in all groups of vertebrates from Pisces to Mammalia. From the foregoing list, it will be seen that herpetomonads in nature have a similarly wide distribution in vertebrates, having been found in the blood of representatives of all the great groups of vertebrates except Amphibia, in which they will doubtless sooner or later be detected. However, it should again be pointed out that in no case in vertebrates is the flagellate form numerous, the leishmaniform phase being the one most seen, and relatively few vertebrates seem to be infected. In some cases it may be necessary to culture the blood in order to detect the parasite. Artifically induced herpetomoniasis resembles visceral leishmaniasis in its insidious onset and pathogenic effects such as feverish attacks, splenic enlargement often accompanied by hepatic enlargement, emaciation, progressive anemia and leucopenia.

The mode of entry of the herpetomonads into the blood of the Dentex examined by us is, unfortunately, not certain. It may be due to the inoculative action of an ectoparasite such as a leech. In the case of fresh-water fishes, herpetomonads might be introduced into their blood by aquatic biting Hemiptera such as Nepa. The entry of a natural intestinal parasite into the blood from the gut seems to be excluded, as, in every case, we carefully examined the gut contents of the fishes dissected by us, but found no indication of the presence of a Herpetomonas as a natural parasite in the guts. We may remark here that we always made a practice of examining before use the

dejecta and blood of the vertebrate animals subsequently used by us in our previous experiments on induced herpetomoniasis, and on no occasion did we find a Herpetomonas occurring naturally in the gut of the vertebrate. On one occasion, in the cloaca of two specimens of *Lacerata vivipara,* we found a uniflagellate monad, but it lacked a blepharoplast, and hence was not a Herpetomonas. Bayon (1915) states that he found a Herpetomonad in the cloaca of a *Chameleon pumilus* on Robben Island, while M. Léger (1918) states that he found a herpetomonad in the rectum of a lizard, *Anolis* sp. in Martinique. It is possible that these herpetomonads in the hind gut of lizards may have been acquired from ingested infected flies, and have passed thence into the blood of the lizards. It is also possible that in the catching of the fly on the tongue of the lizard, herpetomonads may have been liberated from the insect and have passed through the mucous membrane of the tongue of the vertebrate. On the other hand, they may have been inoculated into the blood of the lizard directly by the action of a bloodsucking fly or other Arthropod. We do not consider, on the evidence available, that herpetomonads are natural parasites of the gut of vertebrates, though they may be acquired from invertebrates by way of the gut and pass therefrom into other organs.

In our experience, when a herpetomonad is introduced into a vertebrate host, it may be able to exist either as a somewhat heavy infection that tends to die out and hence is transitory in the vertebrate, or it may become established in so attenuated a form that the pathogenic effects of its presence are not detected unless the resistance of the host is suddenly diminished. Again, owing to periodicity of multiplicative periods of the parasites, they may only be capable of detection in the blood of the host at certain seasons. The accidental, successful introduction of herpetomonad parasites by the agency of certain insects may thus afford an explanation of sporadic outbreaks of such diseases as kala-azar or other form of leishmaniasis.

When a herpetomonad gains access to and proves capable of multiplying in a vertebrate, though the infection may prove to be sparse, as in the case of *H. denticis,* it indicates that while there is still difficulty for the herpetomonad to live in the blood of the vertebrate, yet an attempt is being made that may become more successful—and perhaps more obvious—in the future. In other words, the presence of a natural herpetomonad in the blood and organs of Dentex indicates a further example of the habituation of a flagellate of invertebrates to life in a vertebrate host.

The herpetomonads, indeed, show wonderful powers of adaptation, one of the most plastic being *H. davidi,* natural to the gut of certain plant-frequenting insects, which is able to live in the latex of certain Euphorbiaceous plants.

Such diseases as leishmaniases in vertebrates need not be regarded as necessarily being conveyed by any one specific insect carrier of herpetomonads, but as being transmitted more or less accidentally into a susceptible subject by the agency of any insect capable of being heavily parasited with herpetomonads, and of passing these flagellates into vertebrates.

The leishmaniases are herpetomoniases in which the dominant stage of the causal agent in the vertebrate is the rounded, resting, non-flagellate leishmaniform stage. The leishmaniases are allied in causal agency, pathology and treatment (by tartar emetic) with the trypanosomiases, wherein the dominant stage of the causal trypanosome in the vertebrate is the flagellate stage, but resting, non-flagellate, leishmaniform parasites also occur in the internal organs of the vertebrate.

The occurrence of natural herpetomonads in vertebrates, and the ability to infect vertebrates experimentally with herpetomonads—entailing pathogenic results resembling leishmaniasis in the case of warm-blooded hosts—present an interesting chapter in the evolution of disease.

SUMMARY

A new flagellate, *Herpetomonas denticis,* n. sp., occur naturally in the blood of fish, *Dentex argyrozona,* from St. James, near Cape Town. The parasite was also seen in spleen, liver and kidneys of the fish. Flagellate forms, 5 to 24μ long, and 1.5 to 2.5μ broad, occurred in the heart blood, and rounded, non-flagellate, leishmania-like forms were seen in the internal organs. Multiplication stages were found.

As far as known, this is the first record of the natural occurrence of a Herpetomonas in the blood and internal organs of fishes. Four Dentex, out of 41 examined, were scantily parasited. Herpetomonads were not found in the digestive tracts of the fish.

The significance of this piscine parasite is important, in view of the numerous experiments carried out by the authors and others on the successful infection of vertebrates with herpetomonads and their relation to Leishmania. The leishmaniases are really herpetomoniases of mammals, wherein herpetomonads—which are natural parasites of invertebrates, such as insects—have been introduced into vertebrates, such as mammals, with pathogenic effects.

REFERENCES CITED

Bütschli, O. 1884.—Protozoa. Brown's Kl. u. Ord. d. Tierreiches, vol. 3.

Fantham, H. B., and Porter, A. 1914.—Some Insect Flagellates introduced into Vertebrates. Proc. Camb. Philosoph. Soc., 18:39-50.

1915.—On the Natural Occurrence of Herpetomonads (Leptomonads) in Mice. Parasitol., 8:128-132.

1916.—The significance of Certain Natural Flagellates of Insects in the Evolution of Disease in Vertebrates. Journ. Parasitol., 2:149-166.

Franchini, G. 1913.—Un nouveau protozoaire parasite de l'homme provenant du Brésil. Bull. soc. path. exot., 6:156-158.

Laveran, A. 1917.—Leishmanioses. Paris. 521 pp.

Léger, M. 1918.—Infection sanguine par Leptomonas chez un Saurien. C. R. soc. biol., 81:772-774

1919.—Hématozoaire flagellé nouveau dans une pyrexie cliniquement non classée. Bull. soc. path. exot., 12:80-84.

Explanation of Plate III

Fig. 1.—Photomicrograph of the flagellate form of *Herpetomonas denticis* in the blood of *Dentex argyrozona,* obtained by using Zeiss 4 mm. apochromatic objective and Huyghenian 2 ocular

Fig. 2.—Photomicrograph of flagellate *H. denticis,* obtained by using Zeiss 1/12" oil immersion objective and Huyghenian 2 ocular.

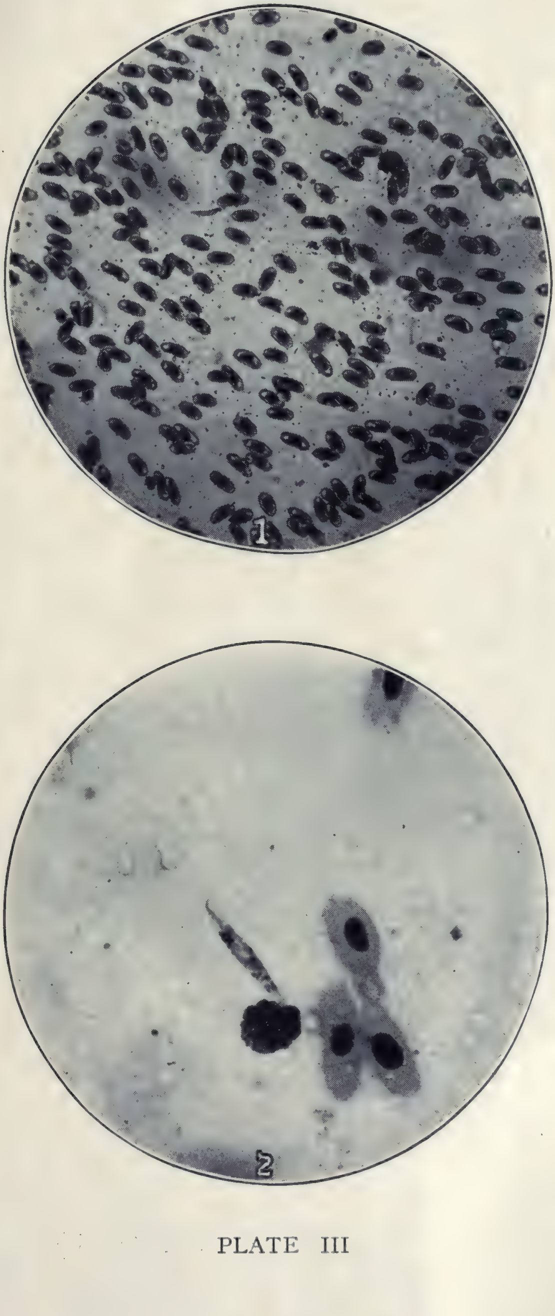


PLATE III

THE DEVELOPMENT OF GREGARINES AND THEIR RELATION TO THE HOST TISSUES: (III) IN *GREGARINA RIGIDA* (HALL) ELLIS

Minnie Watson Kamm

This paper is the third of a series in which it is desired to show the effect of gregarines upon the host cells which they parasitize. In the two former papers the species considered were intracellular parasites, the young stages of which live within the intestinal cells and absorb their entire nourishment therefrom; they are consequently disastrous in their effect upon these cells. In *Cephaloidophora delphinia* (Watson) Kamm (1918) the minute trophozoite soon outgrows the parasitized cell and absorbs the walls of this and the adjoining cells, and finally comes to occupy considerable space within the epithelium, being highly deleterious in its action upon the host tissue. A moderate infection with this parasite therefore causes a serious gregarinosis in the host. It is questionable whether the host, the large white sand-flea *Talorchestia longicornis* (Say), is able to regenerate new tissue to replace the lost.

The first parasite studied (Kamm, 1917), *Stenophora lactaria* Watson, injures its host the milliped *Callipus lactarius* (Say), in much the same manner but to a less serious degree.

The present species, *Gregarina rigida* (Hall) Ellis, parasitizes species of the Acridiidae, the present specimens being taken from *Melanoplus differentialis* (Uhler). It is a particularly good species for sectioning because the percentage of infection runs high and the number of parasites per host is large.

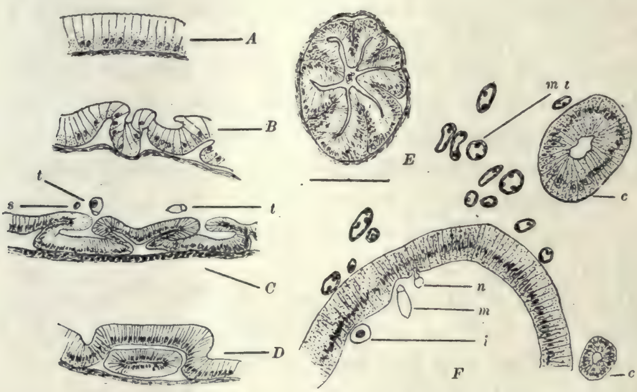
The entire alimentary tract (Fig. 1) was sectioned in order to determine the histology of the cells in the various regions and the degree of parasitism involved, this degree being dependent upon the character of the cells. The walls of the crop are chitinous and armed with teeth and are therefore not adapted to parasitism. The thin-walled, highly vascular mid-intestine is, on the contrary, well adapted to the existence of gregarines; I have found as many as fifty-four parasites in one cross-section made through the anterior portion of this region.

The six pyloric ceca originate near the anterior end of the mid-intestine (Text figs. A-F) and pass both forward and back, the backwardly-directed portions being only about one-third as long and much more slender than those directed anteriorly. They are admirably adapted to the early requirements of the parasite. The tiny sporozoites find lodging here without danger of currents of food or strong mus-

cular contractions tearing them loose from their hold on the cells. Some unilocular stages are to be encountered in the mid-intestine walls but by far the larger number is situated here. Consequently the larger epimerited trophozoites and smaller sporonts are abundant here also.

The Malpighian tubules which open into the alimentary tract at the junction of mid-intestine and intestine proper are not parasitized with gregarines.

The anterior end of the intestine proper is similar histologically to the mid-intestine and contains some gregarines but the rectum, which is chitinous-walled, is free.



Text Figure A-F.—*A, B, C,* and *D* represent the origin of the ceca at the junction of crop and mid-intestine. *A,* cell wall near posterior end of crop. *B,* convolutions in wall. *C,* double folds in wall, cells small, several gregarines near folds, *t* small sporonts, *s* sporozoite, moving freely from *c* ceca to intestine. *D,* cecum closed off from intestinal epithelium but still within longitudinal muscular layer. It soon penetrates this wall and lies freely in the coelom. *E,* cross-section through pyloric orifice of host between crop and mid-intestine. *F,* cross section through anterior end of mid-intestine and through the small backwardly-directed ceca (*c*) and the anterior end of the Malpighian tubules (*mt*); one trophozoite (*n*) is attached to the cell-wall, one small sporont (*m*) is cut longitudinally and a larger one (*i*) crosswise.

All drawings were made at same magnification, the line (under *E*) representing 200μ.

Sections were cut thin (5μ) so as to include portions of the same young parasite in several successive sections. Stains used included Delafield's and iron hematoxylin countered with orange G; best results were obtained with the early stages by using the latter but for the larger parasites the former was the more satisfactory. In order to determine the effect of the parasite upon cells, both stains were useful, the iron stain for the chromatin and Delafield's for cytoplasmic modifications, if any.

Free sporozoites (Fig. 2) were found in large numbers in both mid-intestine and ceca. Much to my surprise they are spherical or sub-spherical in shape, measuring 14 to 20μ in diameter, the nucleus being proportionately large and filled with scattered granules of chromatin.

Upon coming in contact with the epithelium, there is pushed out from the sporozoite a small conical protuberance (Fig. 3) at first devoid of endoplasm. This papilla elongates into a slender neck, often as long as the sporozoite itself, and forces itself through the intima into the wall of the intestine. This spherical sporozoite and its ameboid character have not to my knowledge been heretofore reported for gregarines but from many hundreds of observations with high power, I am convinced that these phenomena exist. The protuberance has been seen in its incipiency through the gradually growing stage when the sporozoite lies adjacent to the wall and to the penetration of the long, now pyriform, trophozoite which becomes firmly attached.

In none of the observations made was the cell-wall punctured, the sporozoite pushing up between two cells and splitting them apart instead. This is obviously the easier process for the apex is unarmed and yet it affords a holdfast, which is all the young parasite desires. The point often enters the cell area at considerable slant and the parasite is unable to recover its normal perpendicular position, possessing a crooked epimerite during the rest of its trophozöitic life.

Sporozoites are generally found in the recesses between folds of the ceca where they collect in groups and are fully protected. They also sink into recesses which appear to have been made by parasites which have left their holdfasts and become free-living sporonts. The more exposed regions are more infrequently parasitized because the sporozoites which have endeavored to attach themselves here have been swept away.

Very many faintly-staining bodies the size and shape of sporozoites are often found massed along the periphery of the lumen, their nuclei generally disintegrated and their outline often indefinite. I venture the suggestion that these are dead or infertile sporozoites; and that sporozoites must be eaten by the proper host within a certain time, probably a few weeks after extrusion from the cyst, in order to remain fertile. The similarity in size and shape preclude the possibility of their being sporozoites of other gregarines not infective in this particular host.

When the process of the sporozoite is first thrust into the cell-area it is ectoplasmic but it soon fills with endoplasm and swells into a knob-like holdfast not easily dislodged (Fig. 5). A septum begins

to develop although not at first visible but evident from the differential staining. The trophozoite has now fully established itself and is growing rapidly. It has crowded several cells aside at their apices, not only the epimerite but a goodly portion of the protomerite being pushed up into this cell-area.

An instance in which the entire trophozoite is pushed up into the cell area is described by Léger and Duboscq (1899) for *Gregarina davini* Léger and Duboscq. The parasite has located in a recess between two lobes, its large dilated epimerite occupying the regenerative space at the base of the epithelium, which contains many nuclei but no cell walls. It transforms this space into "un kyste epithélial." The nuclei are massed near the epimerite but are not in the least affected by its presence.

The septum is now established and the staining in the two parts that are characteristic of the sporont; the nucleus is very large and contains many karyosomes which later fuse into one; and the epimerite now contains a small amount of endoplasm only near its base.

If the epithelium were being affected by the parasite this would now be evident. The cells are undoubtedly crowded and their nuclei often pushed out of place and shape; but upon careful observations with both stains I can detect no difference in character between the normal and crowded cytoplasm or between the normal and distorted nuclei. There is no hypertrophy and I do not think the crowding could be called atrophy for the cells return almost to their normal shape. The larger the parasite becomes the less its presence disturbs the cells, for the body retreats into the lumen except for the small epimerite (Figs. 6, 7). I think the cell always retains a trace of the indentation made by the epimerite and that this depression is taken advantage of by the young sporozoites as a new holdfast.

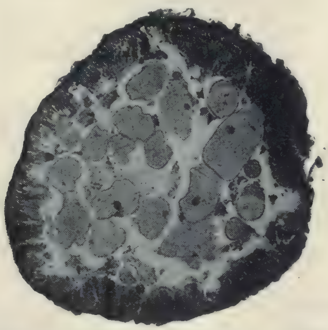
The epimerite is gradually constricted as the parasite grows, until it becomes a perfect sphere, completely pinched off, leaving a small indentation but no open wound in the apex of the protomerite (Fig. 9) which soon smooths out. Vigorous muscular contractions of the intestine or the current of food carries the sporont away and leaves the epimerite in the cells (Fig. 10), where it soon atrophies and disappears.

If its movements are too strenuous or outside mechanical means interfere, the parasite may lose its epimerite prematurely (Fig. 11). I doubt if it is able to recover the loss; it is probable that a thin stream of protoplasm continues to flow from the apex until the protomerite collapses and the animal dies. The parasite is probably unable to regenerate a new epimerite and a new fully developed organ would be unable to penetrate the epithelial cells.

Large free sporonts in the ceca are the exception rather than the rule. A maximum length of 90μ was observed here, the animals soon moving out into the intestine, where more food is to be found. This migration is not due to any new chemical affinity which the parasite acquires, for the weak acid secreted by the gastric ceca passes directly into the mid-intestine, where the parasites continue to be bathed in it; the migration is unexplainable. The fact that the ceca become too small is not adequate explanation.

In the intestine the sporonts are tightly compressed between food-masses the greater part of the time, since the host eats frequently, and they are often flattened so that in cross-section one dimension is three times the other. The parasites just as readily adhere to food masses as to the intestinal walls, which means that they are frequently carried to the exterior in food-pellets.

Two sporonts in association preparatory to the formation of a cyst are shown in the microphotograph (Text fig. G).



Text Figure G.—Microphotograph of section through mid-intestine showing lumen almost completely filled with parasites cut at various angles, the short wavy outlines denoting movements when fixed. Four associations of adult sporonts are shown.

In comparison with the number of parasites encountered, the number of cysts is very small, so the gregarines which are able to complete their life-history are comparatively few in number.

One more paper will be presented in this series, on another epimerited species of a genus not closely related to the genus *Gregarina* and a bibliography will be given covering work on Effect on Host Tissues.

SUMMARY

1. Ten successive stages in the life-history of *Gregarina rigida* (Hall) Ellis are shown from the spherical sporozoite free in the lumen of the intestinal tract to the associative sporonts.

2. This species possesses an epimerite which develops in the sporozoite as an ameboid papilla, becoming a long slender neck, which is thrust through the intima between two epithelial cells rather than into one, where it obtains a holdfast and develops at the cell-apex a rounded knob for an epimerite.

3. The cells parasitized are affected only mechanically, being pushed aside during the parasite occupancy; no chemical effect upon the cell is noted at any stage of occupancy and the cell is apparently uninjured.

Bibliography

Kamm, Minnie Watson. 1917.—The Development of Gregarines and Their Relation to the Host Tissues: (I) in *Stenophora lactaria Watson.* Jour. Parasit., 3:124-30.

1918.—The Development of Gregarines and Their Relation to the Host Tissues: (II) in *Cephaloidophora delphinia* (Watson). Jour. Parasit., 5:35-40.

Léger, L., and O. Duboscq, 1899. Notes biologiques sur les Grillons. C. R. Soc. Biol., (3) 7:xxxv-xl

Explanation of Plates

All drawings except Fig. 1 were made with camera lucida from sections cut at 5μ. The line in drawings 2, 3, 4, and 5 represents 10μ, in the other drawings 100μ.

Explanation of Plate IV

Fig. 1. Alimentary tract of *Melanoplus differentialis* (Uhler), after Folsom.

p pharynx	*m* stomach or mid-intestine
o esophagus	*mt* Malpighan tubules
gl salivary glands	*i* intestine
a crop	*j* colon
g gastric ceca	*r* rectum

Fig. 2.—Two sporozoites free in cecum.

Fig. 3.—Sporozoite with apical protuberance developed ready for penetration into cell area. See also Fig. 13.

Fig. 4.—Portion of cecum with three sporozoites in the process of entering the cellular area, two sharply pointed and one with blunter apex. One sporozoite with elongated protuberance lies near the epithelium. See also Figs. 12 and 13.

Fig. 5.—Slightly knobbed epimerite in young trophozoite in which a difference in staining reaction is discernible between protomorite and deutomerite.

Fig. 6.—A large trophozoite crowded between two lobes of the cecum.

Fig. 7.—A large trophozoite in epithelium with its epimerite somewhat eccentric.

Fig. 8.—Trophozoite with epimerite and septum developed. The epimerite is held in place by pressure from the outside rather than by a specialized holdfast.

Fig. 9.—Sporont with indentation at apex where epimerite has recently become detached.

Fig. 10.—Sporont which has just become constricted from its epimerite.

Fig. 11.—Trophozoite which has become prematurely severed from its epimerite.

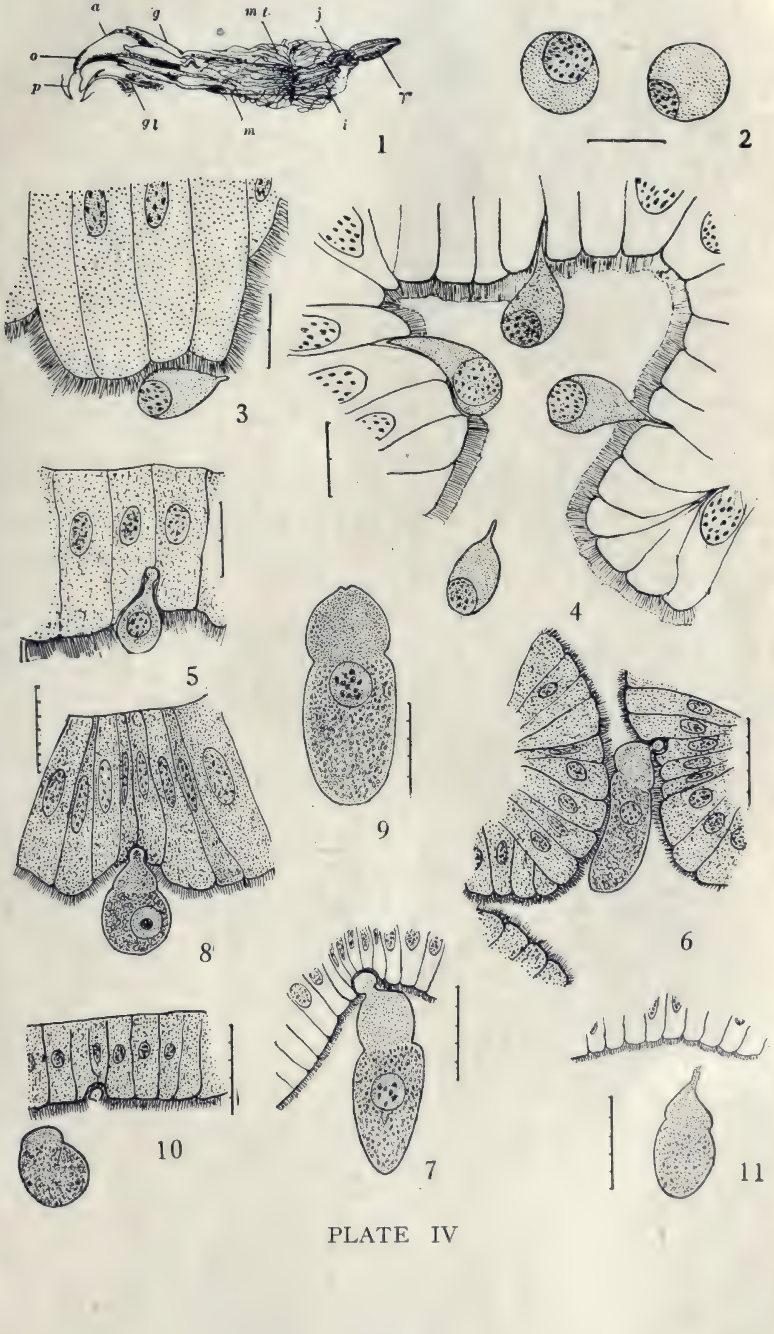
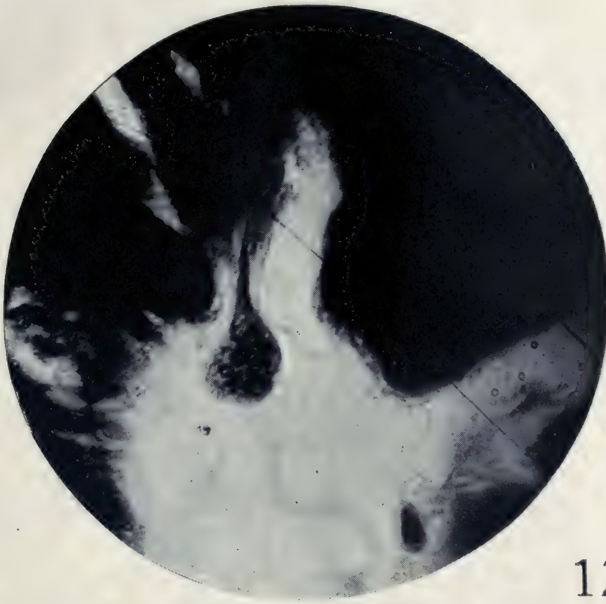


PLATE IV

Explanation of Plate V

Fig. 12.—Microphotograph ($\times$ about 1200) showing pyriform sporozoite about to penetrate into area between cells. Note large nucleus upon face of cell.

Fig. 13.—Microphotograph ($\times$ about 1200) showing three sporozoites, one at right, with protuberance half developed, lying near epithelium; other two with apical ends at lower focus than body of cell but showing the rotund character of the sporozoite at its distal portion, and the large nucleus which contains many distributed karysosomes.



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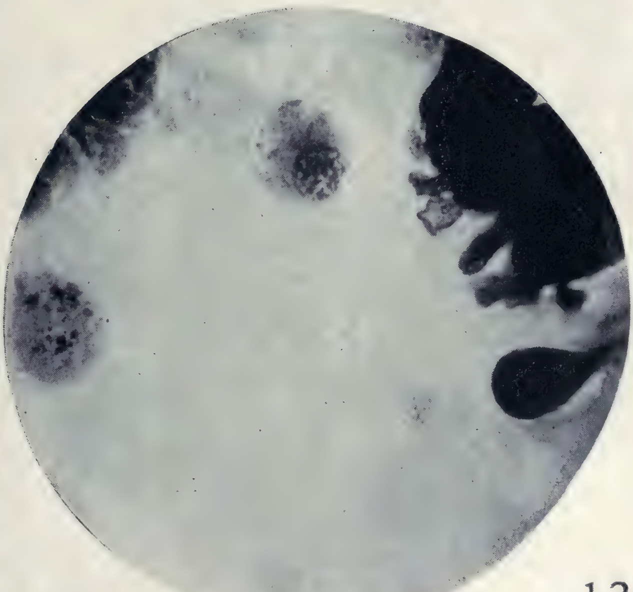

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PLATE V

A NEW NEMATODE FROM THE RAT *

Sadamu Yokogawa

FORMOSA MEDICAL COLLEGE

During the winter of 1920, while I was working at Johns Hopkins University, Dr. W. W. Cort assigned me for investigation the problem of the nematodes of rats and gave me some nematodes, which he had collected from a Norwegian rat, *Epimys norvegicus,* caught in Baltimore. These worms were found to be a new species of Heligmosomum, to which I give the name *Heligmosomum muris* nov. spec. Later I found this same species in twenty-four out of twenty-six rats examined, which were caught near Baltimore. Other nematodes found were *Strongyloides papillosus, Trichosomoides crassicauda, Hepaticola hepatica* and *Heterakis spumosa,* but the present paper describes only *Heligmosomum muris,* since the others have been described by other authors.

Although *Heligmosomum muris* is a common parasite of rats here, nobody seems to have found it previously. My studies on the structure of this species were made for the most part from living material, although specimens preserved in alcohol were used for comparison. The measurements of the body were made on fixed material, because the size of the living worms is changed by movement. Accordingly the sizes given in this paper are a little smaller than those of the living worms.

Specimens of *Heligmosomum muris* preserved in alcohol are dark brown in color, filiform and coiled irregularly two to five times. These worms live in the upper part of the small intestine, especially near the duodenum of the rats. When still in position in the intestine, the worm appears as a little curved red string in the mucus or buried slightly in the mucous membrane. If, however, cool normal saline is poured on the intestine or the rat is not dissected soon after it has been killed, the worms will always be found more or less coiled. In the living worm, the body is red, filiform, and somewhat narrowed anteriorly. The head is small, 21 to 25μ in diameter exclusive of the surrounding cuticular expansion, and 30 to 36μ in diameter including it (Fig. 1). The mouth is small and a small oral cavity (a) is present. In an optical section of this region in worms preserved in alcohol the sub-cuticular part of the circumoral area gives somewhat the appearance of two small teeth, one on each side. The esophagus is conoidiform, a little sinous and 0.35 to 0.45 mm. long. The cuticula has transverse striations and prominent longitudinal markings in the form

* This paper is a contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

of ten ridges. It is relatively thick and inflated on the head and the anterior cervical region, making a cephalic area or expansion. The length of this area is 0.06 to 0.07 mm. The longitudinal cuticular ridges originate a little behind the cephalic area and run parallel clear to the posterior end. The transverse striations are not continuous around the cuticula but are found only on the longitudinal ridges. The excretory system is well developed and takes the form of two elongate sacs, which contain a large amount of highly refractile granules. These sacs are situated on each side of the body and extend to about the middle of the body. The excretory sacs are connected with the excretory pore by a small canal. The excretory pore is situated on the ventral surface, at a distance of 0.8 to 0.14 mm. in front of the base of the esophagus. The nerve ring is situated just in front of the excretory pore at a distance of 0.2 to 0.25 mm. from the anterior end of the esophagus. Cervical papillae are not present. The cells lining the intestine contain a large amount of melanin-like pigment. This is distributed throughout the entire length of the intestine.

The male of *Heligmosomum muris* (Fig. 2) is smaller than the female, being 3 to 4 mm. in length with a maximum thickness of 0.085 to 0.1 mm. at the middle of the body. The bursa (Fig. 3) is large and a little enrolled, expanding toward the ventral side. It consists of two large lateral lobes and a small dorsal lobe. The lateral lobes are each supported by six rays and are asymmetrical, the right being larger than the left. In one worm measured, the right lobe was 0.218 mm. long and 0.164 mm. wide, and the left lobe was 0.164 mm. long and 0.146 mm. wide. The rays of the left lobe are more divergent than those of the right, and are different in form on the two lobes. The ventro-ventral, latero-ventral, externo-lateral and medio-lateral rays of the left lateral lobe are similar in form and have almost equal intervals between them. The left postero-lateral ray has a very different form, being shorter and wider than the others and diverges from the medio-lateral ray, curving posteriad. The postero-lateral ray of the right lobe is relatively small and diverges from the medio-lateral ray, curving a little posteriad. The medio-lateral and externo-lateral rays of the right lobe are digital and larger than the other rays. They run close together and parallel throughout most of their extent, but their tips diverge. The latero-ventral ray of the right lobe is long and straight and the right ventro-ventral ray is slender and quite divergent. Both externo-dorsal rays (*ed*) are thin and slender. They diverge from the root of the dorsal ray and run along the boundary line between the two lateral and the dorsal lobes, curving posteriad; consequently they sometimes appear to belong to the lateral lobe and sometimes to the dorsal. The dorsal lobe is very small and divided from the lateral lobes by

shallow indentations. The dorsal ray (*d*) is 45 to 54μ long and terminates in four digitations.

The body of the male terminates posteriorly in a thin cone which projects into the bursa along the anterior surface of the dorsal lobe. The two spicules are yellowish brown, about 0.56 mm. long and filiform. They are united at their distal ends and form a small arc. The two gubernacula are colorless and situated on the ventral and the dorsal sides of the distal end of the spicules. The ventral gubernaculum is longer than the dorsal. The former is 0.06 to 0.07 mm. long and the latter 0.04 to 0.05 mm. long.

The anterior part of the testis (Fig. 2, *c*) is situated on the dorsal side near the beginning of the intestine and is loop-shaped. Its beginning is very difficult to see because it is covered with the large excretory sac. However, I could see it sometimes clearly in the living worms, and found that the distance of the anterior tip of the testis from the base of the esophagus varied somewhat in different worms. At the middle of the body, a narrow region (*d*) 0.02 to 0.03 mm. long follows the testis. The wall of this region is thick and consists of cuboidal cells, similar to the cells of the ejaculatory duct. It seems to be a vas deferens because it is joined immediately to the seminal vesicle (*e*) filled with spermatozoa. The seminal vesicle is 0.1 to 0.13 mm. long and is followed by a narrow canal 0.09 to 0.12 mm. long which is surrounded by high columnar cells (*f*). Each of these cells contains a round nucleus and many granules. The cells which are situated on the posterior half of this canal are darker than those of the anterior half. These cells probably correspond to the cement glands of other forms, and the darker coloring appears to be connected with the secretory activity of the cells. This part connects with a well developed ejaculatory duct. The wall of the ejaculatory duct (*g*) consists of a layer of transparent cuboidal cells. At the level of the cement gland the reproductive tube crosses the intestine again. Consequently the anterior half of the intestine is located on the ventral side and its posterior half on the dorsal side of the reproductive tube. The distal end of the intestine joins the posterior end of the ejaculatory duct.

The female (Fig. 4) of *Heligmosomum muris* is larger than the male. It is 4 to 6 mm. long, with a maximum thickness of 0.09 to 0.12 mm. in the middle of the body. In the contracted worm the posterior region of the body is withdrawn to such an extent that the cuticula forms a sac surrounding the anus and vulva. The posterior end of the body becomes reduced suddenly in size just behind the vulva, terminating in a short, thin tail. The anterior end of the ovary (*h*) bends and forms a small loop. This part is situated on the dorsal side of the anterior part of the intestine at a varying distance from the base of the esophagus. The blind free anterior

end of the ovary contains extremely small cells, the primordial germ cells. The main part of the reproductive organ is situated on the dorsal side of the body and the ovary is filled with developing oocytes, which generally arrange themselves in single file. The ovary connects with the receptaculum seminalis by a narrow tube near the posterior part of the body. The receptaculum seminalis (*i*) is situated at the beginning of the uterus without sharp demarcation. The uterus is situated in the posterior part of the body and is 0.45 to 0.60 mm. long, containing 13 to 27 eggs, and connects with the ovejector (*j*) of about 0.1 mm. in length. The ovejector is joined to the thick walled vagina, crossing the distal end of the intestine. The ovejector has a well developed wall which at the beginning of the ovejector is thickened forming a sphincter. The ovejector seems to be a little twisted and its distal end projects into the vagina. The vagina (*k*) is 0.14 to 0.16 mm. long and situated on the ventral side of the body. Its wall is lined by cuticula. There are strong irregular longitudinal folds on its internal surface and it extends into the distal end of the ovejector. The vagina runs in a diagonal direction in the posterior end of the body, and terminates in the vulva. This (*l*) is situated on the ventral surface just in front of the anus at a distance of 0.1 to 0.13 mm. from the tip of the tail. The intestine is straight and runs along the ventral side of the body, terminating in the anus. It crosses the ovejector near the posterior end of the body. The anus (*m*) is situated at a distance of about 0.06 mm. from the tip of the tail. The eggs are ellipsoidal with a very thin shell. The average size is 58μ by 33μ; a common minimum is 54.6 by 30.9μ and a frequent maximum 61.8 by 34.5μ. They are in the one to sixteen cell stages of development in the uterus and in the feces are in the four to sixteen cell stage, exceptionally, also in the morula stage.

In the genus Heligmosomum, Raillet and Henry the following species have been placed: *Heligmosomum costellatum* (Dujardin), *H. minutum* (Dujardin), *H. gracile* (F. S. Leuckart), *H. laeve* (Dujardin), *H. braziliense* Travassos, *H. agoutii* Neiva, da Cunha and Travassos, *H. vexillatum* Hall and *H. cristatum* Gedoelst. All of these are discussed by Hall (1916: 149-158) except *H. agoutii* Neiva, da Cunha and Travassos (1914) and *H. cristatum* Gedoelst (1917). Seurat (1915) gives a detailed description of *H. laeve* Dujardin, which is not fully covered in Hall's paper. *Heligmosomum muris* is most closely related to *Heligmosomum vexillatum* Hall, which was described from *Thomomys fossor* from Colorado, and to *Heligmosomum brasiliense* Travassos from the Norwegian rat from Rio de Janeiro, Brazil. A comparison of *H. muris* with the description of *H. vexillatum* and with a toto mount of this species which was kindly loaned me by Doctor Hall, showed that while these two species agree in general size and shape, they are strikingly

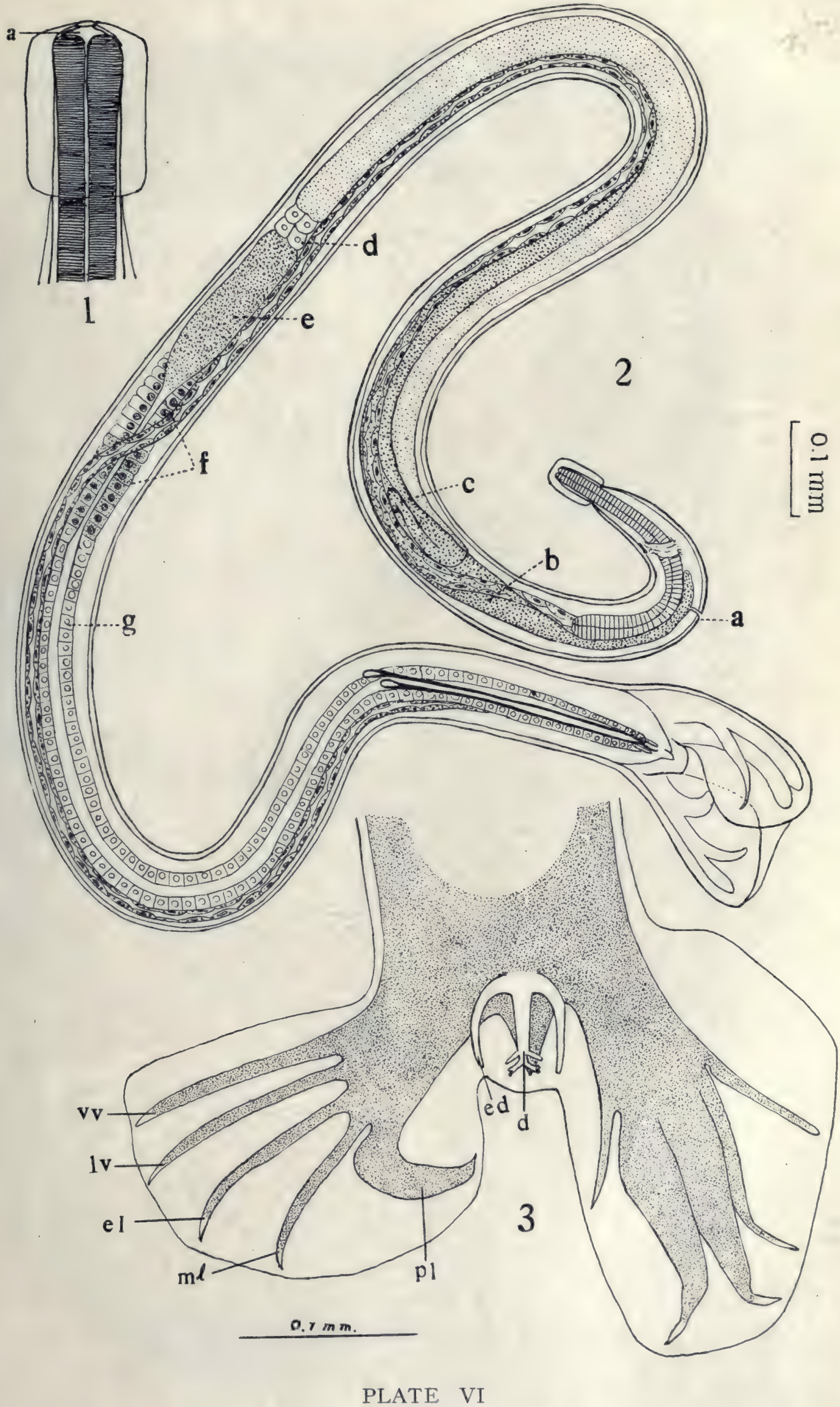

PLATE VI

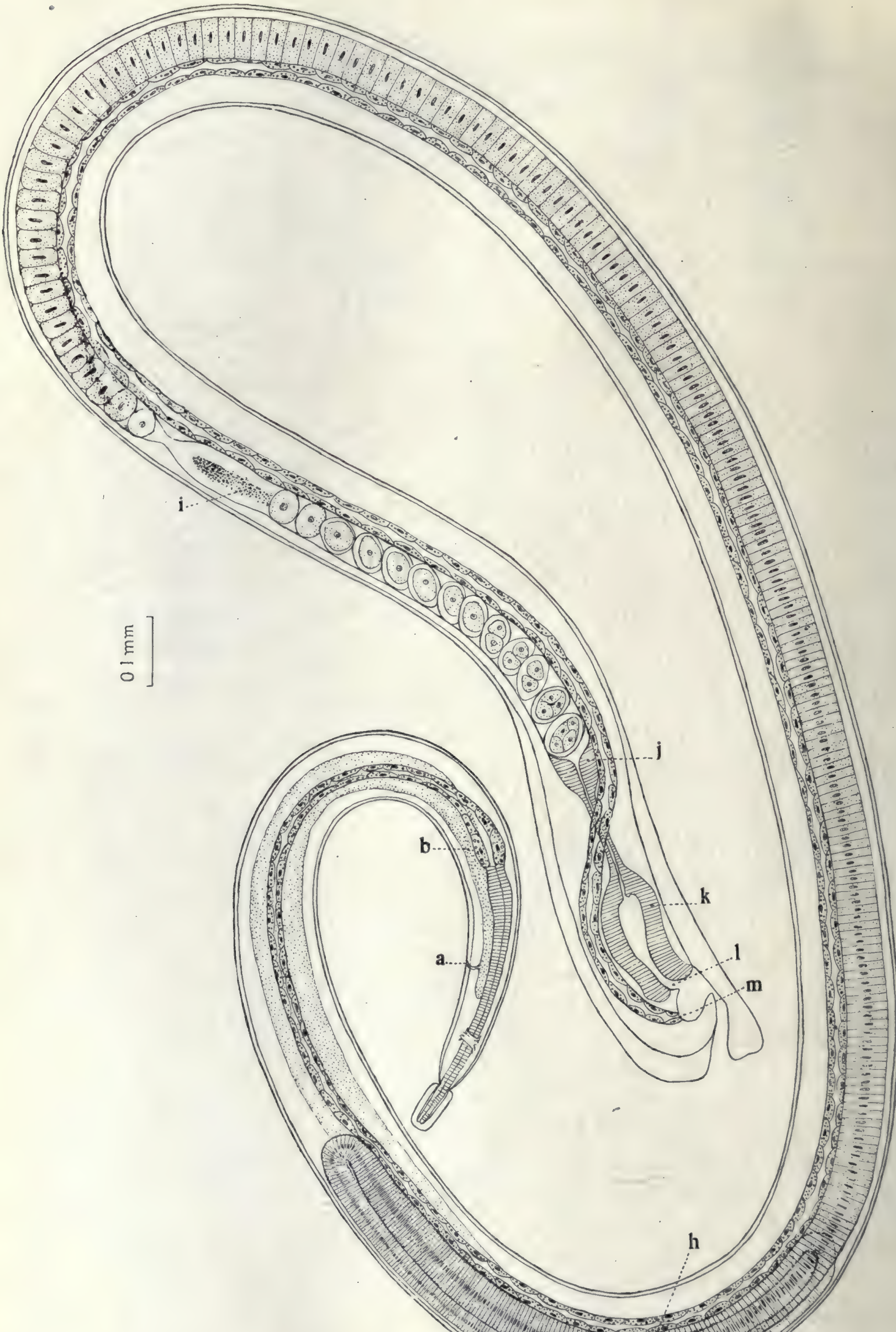

PLATE VII

different in the length of the spicules, the size of the eggs, and the character of the bursae. A comparison of my species with *H. braziliense* is more difficult since this species is not figured and not described in detail, especially in regard to the character of the bursa. Differences are apparent in the size of the eggs, the length of the esophagus, the structure of the bursa, and the position of the anus and vulva. The most striking differences are in the size and shape of the two species. In *H. braziliense* the male is from 2.6 to 2.8 mm. in length and 0.09 to 0.1 mm. in diameter at its widest part, giving the ratio of length to width of about 30: 1. The female is 3.5 mm. long and 0.13 mm. in width with a ratio of length to width of about 27: 1. *H. muris* is longer and especially in the female much narrower for its length, as in this species the male has a length of 3 to 4 mm. and a width of 0.85 to 0.1 mm., making a ratio of length to width of about 33 to 44: 1, and the female has a length of 4 to 6 mm. and width of 0.09 to 0.12 mm., making a ratio of length to width of 44 to 50: 1. These differences seem to me to make it necessary to establish *Heligmosomum muris* as a distinct species.

I wish to express here my deep indebtedness to Doctor W. W. Cort, under whose direction this work was carried on, and also to Doctor B. H. Ransom and Doctor M. C. Hall for their kindness in helping me with the preparaton of this paper.

Papers Cited

Gedoelst, L. 1917.—Nematodes parasites du *Sciurus prevosti* de Sumatra. Rev. zool. Africaine, 5: 153-162.

Hall, M. C. 1916.—Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U. S. Nat. Mus., 50: 1-258.

Neiva A., da Cunha, A. M., and Travassos, L. 1914.—Parasitologische Beitrage. Memorias do Instituto Oswaldo Cruz, 6: 180-191.

Seurat, L. G. 1915.—Sur les conditions de la ponte du strongle lisse. Bull. scient. France et Belg., 48: 171-177.

Travassos, L. 1914.—Tricostrongylidoes brazileiros. Brazil medico, 28: 325-327.

Explanation of Plate VI

Fig. 1.—The anterior end of *Heligmosomum muris,* shown in optical section; *a,* mouth cavity.

Fig. 2.—Male of *Heligmosomum muris* from an experimental rat, 9 days after infection, latero-ventral view; *a,* excretory pore; *b,* excretory sac; *c,* anterior end of the testis; *d,* vas deferens; *e,* seminal vesicle; *f,* cement gland; *g,* ejaculatory duct.

Fig. 3.—Bursa of male of *Heligmosomum muris,* dorsal view; *d,* dorsal ray; *ed,* externo-dorsal ray; *pl,* postero-lateral ray; *ml,* medio-lateral ray; *el,* externo-lateral ray; *lv,* latero-ventral ray; *vv,* ventro-ventral ray

Explanation of Plate VII

Fig. 4.—Female of *Heligmosomum muris* from the wild rat. *a,* excretory pore; *b,* excretory sac; *h,* anterior end of ovary; *i,* seminal receptacle; *j,* ovejector; *k,* vagina; *l,* vulva; *m,* anus.

A NEW RECORD OF *TAENIA CONFUSA*, WITH ADDITIONAL NOTES ON ITS MORPHOLOGY *

Asa C. Chandler

In a collection of parasitological specimens which the writer recently received from Dr. Mark F. Boyd of the Department of Bacteriology and Preventive Medicine at the University of Texas Medical School, Galveston, there was a specimen of a tapeworm which upon investigation was found to be, apparently, an example of *Taenia confusa* Ward 1895. The worm had been sent to Dr. Boyd from the medical school hospital as a specimen of *Taenia saginata,* and was given to the writer as such, without ever having been more than casually examined. Efforts are at present being made by Dr. Boyd to get some information as to the origin of the worm, but to this date these efforts have not been successful. Nothing definite can be said at present as to its origin except that the patient was probably a native of Texas.

Particular interest attaches to the occurrence of this worm, inasmuch as hitherto only two specimens of *Taenia confusa* have been recorded, both having been sent, at different times, to Dr. H. B. Ward by a physician at Lincoln, Nebraska, in 1895. During the twenty-five intervening years no further specimens have been discovered, yet the present occurrence of a specimen from an individual in Texas indicates a strong probability that the worm has existed throughout this time in the Southern part of the middle western portion of the United States in sufficient numbers to protect it against extermination. It is probable that, as in this case, it may frequently have been passed over as a specimen of *Taenia saginata.*

Although in general agreeing with the description of *Taenia confusa* as given by Guyer (1898), the present worm differs in some details, especially of measurements, though not to such an extent as to throw serious doubts on its identity. It is, however, important to note that this worm largely bridges the gap between *T. confusa* Ward 1895 and *T. bremneri* Stephens 1909. The description of the latter is very meager, but the principal difference between this species and *Taenia confusa,* as far as determined by the few segments from which Stephens wrote his description, is in the greater width of the terminal segments, and in the greater abundance and larger size of the calcareous bodies. In both these respects the present worm is

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* Contribution from the Biological Laboratory, Rice Institute, Houston, Tex.

intermediate between *T. bremneri* and *T. confusa*. According to Dr. Bremner, who sent the specimen to Stephens from northern Nigeria, "All Fullani (a Nigerian tribe) women have them, and they are got thru drinking sour milk." Since many of the American negroes originally came from Nigeria, the occurrence of this worm in the Southern United States would very readily be explained. It is, therefore, proposed that until further evidence to the contrary is obtained, *Taenia bremneri* be considered a synonym of *Taenia confusa*.

Before discussing any of the details of the present worm, a brief account of the general morphology and anatomical peculiarities of *Taenia confusa* as described by Guyer (1898) is in place. *T. confusa* is a tapeworm from 5 to 8 meters in length, consisting of from 700 to 800 proglottids, almost all of which are longer than wide. The terminal proglottids are from 27 to 35 mm. long by 3.5 to 5 mm. wide. The scolex is not certainly known. One of Ward's specimens was provided with a scolex and Ward (1897) states that this scolex was studied by him, still attached to the entire chain, under a lens, and that it was approximately the size and shape of the scolex of a Dipylidium. This head was cut off, stained, and mounted by an assistant. It proved to be so much like the head of a Dipylidium that Dr. C. W. Stiles, according to Ward, stated that it could be nothing else. Ward states that so far as he is aware there was no opportunity for it to be confused with the head of another tapeworm, but on the evidence of the improbability of a Taenia having a head so strikingly like a Dipylidium, he was unwilling to record the head as that of the worm he was studying.

The principal anatomical features of the worm, as described by Guyer, which differentiate it from other human Taeniae are the following: delicate cuticle and musculature; small sparse calcareous bodies; small testes; small shallow genital pore, with plug-like papilla nearly filling it; vagina with distinct receptaculum seminis, preceded by a short, constricted, thick-walled portion, and with cilia doubtful, and if present pointing towards the pore instead of away from it; shell gland oval, traversed by vaginal canal, and connected with uterus by separate egg canal opening into dorsal side of uterus; ovaries large, kidney shaped; vittelaria triangular, unpaired, wedging in between ovarian lobes; ripe uterus with median stem and 14 to 18 irregularly disposed and irregularly ramifying branches, with a series of finger-like branches transversely arranged across the anterior end, the eggs emptying out before disintegration of the segment; eggs oval, $30\mu x 39\mu$, without evident pyriform apparatus.

The general morphology of the present worm agrees in most details with that of Ward's specimens as described by Guyer, the scolex not being considered. The worm here described consists of

approximately 790 segments, the great majority of which are longer than wide. The terminal segments of this worm, measuring from 25 to 33 mm. in length, are from 6 to 8 mm. in width, as compared with a width of from 3.5 to 5 mm. in Ward's specimens, and of 9 mm. in *Taenia bremneri.* The width of the segments which are past sexual maturity but not yet fully ripe increases to about 9 mm., this width being retained for a long distance in segments gradually increasing in length from 9 to 20 mm. The approximately square segments measuring 9 by 9 to 10 mm. agree with Guyer's measurements for segments 150 to 250 cm. back of the head, which are 10 mm. long by 9 to 10 mm. wide. The difference in the width of the terminal segments may very possibly be due to a difference in the state of contraction, especially inasmuch as the worm here described does not have such conspicuously flaring posterior ends on the proglottids.

There are a number of differences between this worm and Ward's specimens in the measurements of organs. Some of the larger measurements of the present worm may be partially accounted for by the fact that the measurements are for sexually mature proglottids which measure about 9 by 9 mm. and in which the uterus is already provided with branches, whereas Guyer's measurements appear to be for the organs as they appear in much smaller segments, with unbranched uterus, which he considered sexually mature, possibly relying too much on analogy with *Taenia saginata* or *Taenia solium.* The genital pore measures from 0.8 to 1.2 mm. across by 0.25 mm. in depth, thus resembling *Taenia saginata* much more closely than do Ward's specimens. The structure of the genital pore region is similar as regards the plug-like papilla which nearly fills it, and at the tip of which the cirrus opens. It differs, however, in that the vagina also opens at the tip of the plug, just posterior to the opening of the cirrus; in fact, there is a very short common opening, about 50μ in depth.

The vagina has the peculiar features described by Guyer. In this specimen the cilia are very distinct and, as suspected by Guyer, point *towards* the genital opening, instead of away from it. Just before entering the lens-shaped receptaculum seminis there is an abrupt reduction in the lumen of the vagina with a much increased thickness of the walls, as described by Guyer. It has not been possible in the new worm to trace out the egg duct from shell gland to the dorsal wall of the uterus, but the uterus does not appear to enter the shell gland directly. The vas deferens is as described by Guyer, much coiled, and ends near the middle of the segment. In a few mounted proglottids the vasa efferentia leaving the vas deferens show very clearly, particularly so in a proglottid represented in figure 1. The ovaries in proglottids in which the uterus is unbranched are

about 1.8 by 0.65 mm. and 1.3 by 0.6 mm. respectively (the segment measures 6.5 by 4.5 mm.) but the segments do not have the reproductive organs of either sex fully matured until they reach a size of approximately 9 by 9 mm., and have the uterine branches already evident. In such segments the larger of the fully developed ovaries measures 2.7 by 1.5 mm. The vitellaria vary considerably from the broad and narrow form shown in figure 1 to a short and wide triangular form as figured by Guyer; the scalloped posterior edge is a constant feature. The ripe uterus is as described by Guyer; the most salient feature is the great irregularity of the short deeply subdivided branches which frequently become constricted at the point of emergence from the main stem; the terminal twigs, on the other hand, are swollen and contiguous. There is a series of forward-projecting finger-like branches at the anterior end, and there are two or three deeply-cleft branches prolonged in a backward direction, the main stem of the uterus not extending back of the shell gland. The type of branching of the ripe uterus is reminiscent of that of *Taenia hydatigena* Pallas. The testes in the present worm measure from 105 to 125μ in diameter, as compared with 89 to 96μ according to Guyer, and 150μ in *Taenia saginata.* A few testes near the junction of the vasa efferentia with the vas deferens are greatly enlarged, and may be 195μ in diameter, as shown in figure 1.

The uterine eggs of the present worm are approximately the size and shape reported by Guyer for Ward's specimens, though the majority are a little larger (33 by 42μ). There is a distinct pyriform apparatus in the form of two short filaments attached to the thin outer shell as shown in figure 2.

The scolex is the most interesting part of the worm here described. Although the scolex was attached to only a small portion of the body of the worm, there seems to be no reason for doubting that the head really belongs to the worm here described. There are no segments of any other worm associated with this one to indicate a double infection, and the breadth of the neck attached to the head is the same as that of the smallest section of the proglottids. Moreover, the head, although unquestionably a Taeniid head, is quite different from that of any other human species of tapeworm. It does not in any way resemble the head of *Dipylidium.*

The scolex is unarmed, and is very sharply demarcated from the neck, as will be seen by reference to figure 3. It is decidedly oblong in shape and has the suckers grouped into a pair on each side.

As stated at the beginning of this paper, in spite of certain discrepancies in measurements between this worm and those described

by Guyer, the anatomical features which this worm has in common with *Taenia confusa* leave little room for doubt that it should be referred to that species.

If, when *Taenia bremneri* becomes better known, it shall prove to be identical with *Taenia confusa*, as there appears every reason to believe is the case, judging from our present meager knowledge of it, there will be little room for doubt but that *Taenia confusa*, like *Necator americanus*, and other noxious parasites, was brought to America from Africa with the slaves.

BIBLIOGRAPHY

Guyer, M. F. 1898.—On the Structure of *Taenia confusa*. Zool. Jahrb., Syst., 11:469-492.

Stephens, J. W. W. 1908.—Two New Human Cestodes and a New Linguatulid. Ann. Trop. Med. and Parasit., 1:549-556.

Ward, H. B. 1896.—Report of the Zoologist, Ann. Report Nebraska State Bd. Agr. for 1895, 257-272.

1896.—A New Human Tapeworm. West. Med. Rev., 1:35-36. Copied in Amer. Monthly Micr. Jour., 18:305-307.
for 1896, 173-189.

1897.—Note on *Taenia confusa*. Zool. Anz., 20:321-322.

1897.—Report of the Zoologist. Ann. Report Nebraska. State Bd. of Agr.

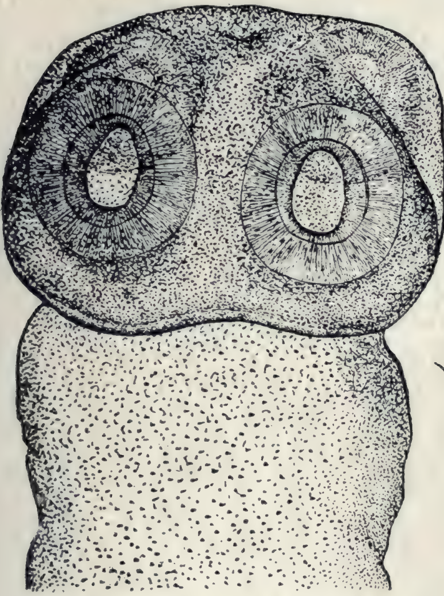
Explanation of Plate VIII

Fig. 1.—Proglottid of *Taenia confusa*, a little past sexual maturity, showing general arrangement of organs, except uterus, which is very indistinct in this proglottid. Note very distinct vasa efferentia, and enlarged deep staining testes near the end of the vas deferens. × 7.

Fig. 2.—Uterine eggs of *Taenia confusa*. × 500.

Fig. 3.—Scolex of *Taenia confusa*, viewed on broad face. × 50.

Fig. 4.—Scolex of *Taenia confusa*, as viewed from anterior end to show oblong shape, and bilateral arrangement of suckers. × 30.



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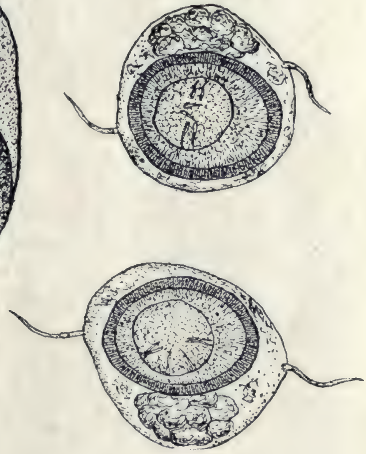

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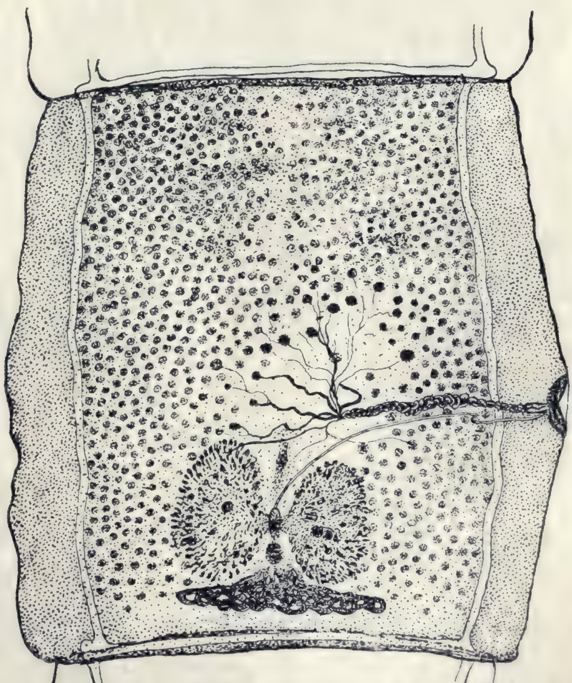

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PLATE VIII

A POSSIBLE INTERMEDIATE HOST OF *FASCIOLA HEPATICA* L. 1758 IN NORTH AMERICA *

MARK F. BOYD

Liver fluke disease of sheep and cattle caused by *Fasciola hepatica* L. 1758 is cosmopolitan in its distribution. According to Hall (1917), in the United States this disease is fairly well established along parts of the Pacific, the Gulf and eastern Atlantic coasts. Francis (1891) has definitely outlined its distribution in Texas. He states that: "This well known parasite occurs in the livers of cattle, sheep and goats of Texas in sufficient numbers to cause great damage. The portion of the state permanently infected consists of the coast counties and river bottoms."

The complicated life cycle of the fluke was first worked out in Europe by Creplin, Weinland, Leuckart and Thomas, and is too well known to require repetition. They found the larval stages to be passed in a small snail, *Limnaea truncatula.* According to Stiles, Leuckart also showed that the redia, but not the cercaria, would develop in *Limnaea peregra.* He also states that Lutz observed that in the Hawaiian islands both *L. oahuensis* and *L. rubella* serve as intermediate hosts. Stiles (1894-95) pointed out that none of these closely allied species of snails are found in America, while fluke disease is found in both North and South America, and concludes that there is either on this continent some other species of snail which may act as intermediate host, or some of the species described in America must be identical with some of the above named forms. He also advanced the view that in North America suspicion would especially fall upon *Limnaea humilis,* Say.

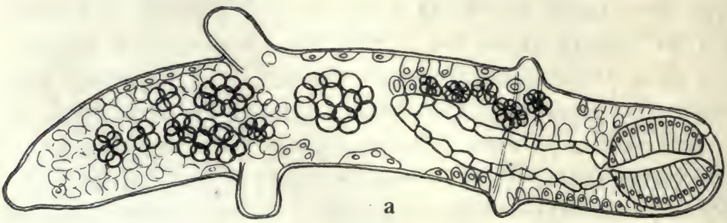
So far as we are aware, observations of the intermediate stages (sporocyst, redia, cercaria) of *F. hepatica* have never been recorded from any snail in North America. *L. humilis,* as suggested by Stiles, has been generally regarded as the intermediate host, but the fact has not been established. In view of the importance of this well known parasite, it seems surprising that this uncertainty regarding its North American intermediate host has not been cleared.

From time to time sheep have been grazed on Galveston island, and fluke disease has appeared among them. It therefore appeared probable that a suitable intermediate host must exist upon the island. Collections of fresh water snails from ephemeral pools on the island

* Contribution Number 4, from the Laboratory of Bacteriology and Preventive Medicine, Medical Department, University of Texas.

revealed the existence of three species, which were identified by Mr. H. B. Hannibal of San Francisco as: *Limnaea humilis,* Say, *Physa fontinalis acuta,* Drap., and *Succinea grosvenorii,* Lea.

Owing to the general view that *Limnaea humilis* is the intermediate host, it appeared that infection experiments designed to clear up this point would be of value. A limited supply of the living ova of *F. hepatica* were secured through the kindness of Prof. A. C. Chandler of Rice Institute, collected from the bile ducts of cattle and sheep at the Houston abattoir. A collection of adult fresh water snails was made on the island in the middle of December and kept alive in an aquarium, with the hope that they would spawn, and provide an adequate supply of young snails. Both *L. humilis* and *P. fontinalis* were represented in the collection. The *Limnaea* died in about two weeks without spawning. On the other hand, the *Physa* survived considerably longer and by Dec. 27 had deposited several masses of spawn.



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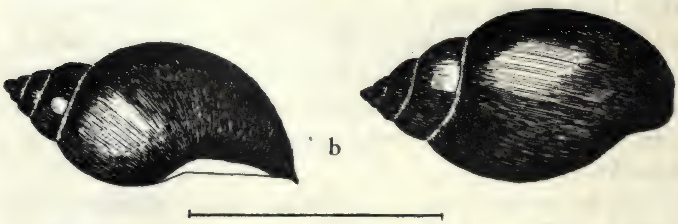

b

Figure a.—Sketch of living redia of *Fasciola hepatica.* Length 560 micra.
Figure b.—Dorsal and lateral view of shell of *Physa fontinalis acuta,* Drap. Length of scale 10 mm.

The egg masses were transferred to a beaker containing tap water and algae, which was kept at room temperature, and were all hatched out by the middle of January. After all the young snails had emerged, a heavy suspension of *F. hepatica* ova was added to the beaker on January 16. At that time there were approximately 100 active young *Physa* in the beaker, about 1 mm. in length. On January 27 it was observed that the majority of the snails were dead and there were less than two dozen survivors. The remainder were examined

at varying intervals for larval stages of *F. hepatica,* by crushing them on a slide under a cover glass in a drop of the aquarium water. The records of these examinations follow:

Date	No. Snails Crushed	Results of Examination
1/27/20	2	Negative
2/ 2/20	2	Negative
2/12/20	3	Negative
2/20/20	1	Found two rediae
2/27/20	3	Found three rediae in one snail
3/10/20	5	Found eleven rediae in one snail
3/12/20	4	Negative

All of the fluke ova employed were apparently fertile, as none were observed in the aquarium by February 12 with the operculum in place, showing that a miricidium probably emerged from each ovum.

The rediae were apparently in the digestive gland of the snail. The appearance of a single redia is shown in figure *a*. They were as actively motile as could be expected, since they were under compression. Contraction and expansion of the body was marked in all, together with contraction and expansion of the muscular pharynx. The blind gut was filled with cellular debris evidently derived from the digestive gland of the snail. When contracted they measured about 450 micra in length, and when expanded, about 560 micra. The space between the cuticle and gut contained several masses of germinal cells. In none were cercariae found.

It is to be regretted that we failed to secure a sufficient number of infected *Physa* so as to prolong the observations to determine whether cercariae would develop and emerge. As it is, the data presented do not enable us to conclude that the larval cycle can be completed in *Physa,* nor settle the question regarding *L. humilis.* Later in the season when we did secure a small brood of *L. humilis* from spawn, we were unable to secure any fluke eggs.

In this connection, some statements by Gilchrist (1918) are of interest. He states that in South Africa the commonest fresh water snail is *Physa* (*Isidora*) *tropica* and that in them are found abundant stages of a fluke very closely resembling those of the liver fluke. He also states that in Australia the intermediate host of the liver fluke is believed to be a species of *Physa.*

References Cited

Francis, M. 1891.—Liver flukes: The common fluke (*Distomum hepaticum*), a new species (*Distomum texanicum*). Bull. Texas Agr. Exp. Sta. 18:127-136, 8 pls.

Gilchrist, J. D. F. 1918.—The life history of *Distoma luteum*, n. sp., with notes on some cercariae and rediae found in S. Africa. Parasitology, 10:311-319.

Hall, M. C. 1912.—Our present knowledge of the distribution and importance of some parasitic diseases of sheep and cattle in the United States. Circ. Bur. An. Ind., 193:419-463, 2 pls.

Stiles, C. W. 1894-95.—The anatomy of the large American fluke (*Fasciola magna*), and a comparison with other species of the genus Fasciola, s. st. Jour. Comp. Med. and Vet. Arch. 15:161-178, 225-243, 299-313, 407-417, 457-462.

Stiles, C. W. 1898.—The flukes and tapeworms of cattle, sheep and swine, with special reference to the inspection of meats. Bull. Bur. An. Ind., 19:11-136, 124 figs.

DIBOTHRIOCEPHALUS TÆNIOIDES LEON, A NEW CASE IN ROUMANIA *

N. Leon

The helminth which forms the object of this note was sent by Doctor Bacaloglou, Professor of Clinical Medicine in our faculty, and accompanied by the following record: "Mr. P., 60 years old, director of a bank, had suffered for two months from gastro-intestinal troubles. He is habitually of good health, not syphilitic, alcoholic, nor given to smoking. The urine at present contains neither sugar nor albumen.

"In spite of the efforts of several physicians, he continued to suffer with vague intestinal pains, and especially a painful morning diarrhea. On some days he had five or six evacuations, accompanied by particles of solid mucus and even of whitish masses like coagulated white of egg. Milk diet to which he was submitted originally, aggravated all these symptoms. I discontinued the milk and put the patient on a diet composed of gruels, soups, and marmalades. I prescribed for him 0.50 cg. of calomel, and following this capsules with betol (1.50 gm. daily with salicylated bismuth). Thereupon he expelled a long fragment of a broad tapeworm. As it did not possess the head, I had him take two days later 6 grams of extracts of male fern. In place of obtaining the remainder of the worm, which perhaps was lost with the fecal matter, the patient expelled a large specimen of *Ascaris lumbricoides*.

"I add that the general condition of the patient, in spite of the gastro-intestinal troubles which had persisted for two months, was good. He had none of the anemia described by authors for carriers of the broad tapeworm — anemia which furthermore did not exist among other patients that I have cared for in that disease. A fortnight after the expulsion of the two parasites, I revisited the patient. He was happy at the disappearance of the diarrhea. The phenomena of mucomembranous entero-colitis are certainly related to the parasites that he harbored."

The worm lacks the head, neck and a good portion of the chain with young rings. The length of the part evacuated is 82 cm., but the widest segments measure hardly 6 mm. The color is ashy yellow, and the segments are very delicate. The form of the segments varies according to age. The youngest, that is to say those closest to the head, are a little broader than long, at most one and one-quarter, but

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never several times broader, as in the case of *Dibothriocephalus latus*. The joints change in a gradual manner into a quadrate type equal in both dimensions. At the end the oldest joints are longer than broad, and all the longer because they are more advanced in age. In comparing this specimen of Professor Bacaloglou's with the specimen that I described (No. 2 in the *Centralblatt f. Bakt., I Abt. Originale,* 1916), I determine that the two individuals are the same species. The

Fig. 1.—*Dibothriocephalus taenioides.* Fragments of chain.

common characteristics which distinguish them from *Dibothriocephalus latus* are not anomalies, as I thought at the beginning, since the anomalies which have been observed among the Bothriocephalids, such as intercalated segments, fenestrated rings, scaly segments, etc., are isolated, or even if they form a portion of the chain, it is relatively

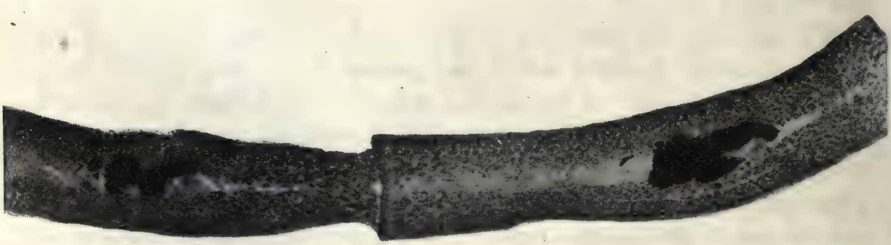

Fig. 2.—End portion of the chain.

very short in relation to the length of the normal worm. The abnormal rings are always situated between other rings of the normal series. The coils of the uterus, which show themselves easily on account of the transparency when they are full of eggs, are two or three in number, and their arrangement takes a characteristic form, bi- or tricornuate (Fig. 3). Even under the naked eye and at a distance one recognizes that the worm is not *Dibothriocephalus latus*. In that species the coils in general number five on each side. They show

themselves under the classic appearance of a rosette, while in *Dibothriocephalus taenioides* they are, as I have said above, bi- or tricornuate.

The characteristics which necessitate the creation of this species under the name of *Dibothriocephalus taenioides* are the following:

(a) *Form of Proglottids.*—The ripe joints are always longer than broad, and the others follow on with the most striking regularity as in *T. solium or T. saginata,* first those a little broader than long; then those which are quadrate and following them such as are longer than broad.

(b) *Size of Segments.*—The largest segments of *Dibothriocephalus taenioides* hardly reach 6 mm., but the major part are narrow as in *Dibothriocephalus parvus* Stephens; yet they differ from segments of the latter in that these, although very narrow, are very short, while the segments of *Dibothriocephalus taenioides,* while narrow, are long.

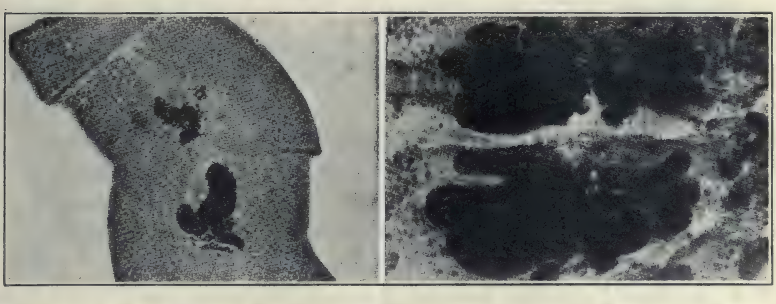

Fig. 3.—At left, *Dibothriocephalus taenioides,* and at right, *Dibothriocephalus latus,* photographed to compare uterine rosettes.

(c) *Form of the Uterine Rosette.*—In *Dibothriocephalus latus* the uterine coils are five on each side, forming the characteristic uterine rosette, while in *Dibothriocephalus taenioides* uterine coils filled with eggs number only two or three.

(d) *Musculature.*—The musculature, both longitudinal and circular, is markedly reduced, so that as a result *Dibothriocephalus taenioides* is very slender.

(e) *The color* is a characteristic ashy yellow.

The preparations are preserved in the collection of the Laboratory of Parasitology of the Faculty of Medicine at Jassy.

A NEW COURSE FOR MIGRATING ANCYLOSTOMA AND STRONGYLOIDES LARVAE AFTER ORAL INFECTION

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The modes of infection and course of migration for Ancylostoma and Strongyloides have been accurately and decisively investigated by various helminthologists old and new; especially recent works by Looss and Fülleborn have decided almost all problems in the subject so fully that no further investigations are needed.

Ancylostoma and Strongyloides larvae are believed to infect in two ways, through the skin and the mouth, the former being the more prevalent manner of infection. The course of the migrating larvae in the host is essentially the same in both cases of skin and of oral infection. Sooner or later they migrate into the lungs by means of blood vessels or sometimes by the lymph system; then the majority of the larvae in the lungs pass through the trachea, esophagus and stomach to the small intestine where they grow into the adult form; but a few of them migrate from the lungs to the intestinal wall by way of the blood vessels, passing through the pulmonary vein, heart and mesenteric arteries, whence they penetrate into the canal of the intestine where they become mature.

This is the universally recognized course of the larvae in the body of the host. In connection with my study on the migration of ascarid larvae in the body of their host, it occurred to me that the well defined course of Ancylostoma and Strongyloides larvae in the host although the principal one, may not be the only one. The following experiments will serve to test not only the new course of migrating Ancylostoma and Strongyloides larvae, but prove definitely my proposal regarding the migration of ascarid larvae.

For the experiments Ancylostoma larvae were cultured from eggs from human feces and filariform Strongyloides larvae were obtained from a culture of eggs from the feces of some monkeys.

Exp. 1. At 5 p. m. on July 17, 1918, two guinea-pigs, A and B, were fed with the Strongyloides larvae cultured for nine days. Animal A was killed at 1 p. m. on the next day, twenty hours later. Two specimens of active larvae were found in the pleural cavity and three in the abdominal, but none in the lungs.

Exp. 2. At 9 a. m. on July 24, a guinea-pig was fed with the Strongyloides larvae cultured seven days and it was killed at noon on the next day, twenty-

seven hours later. Six specimens were found in the abdominal cavity, two in the pleural and two in the left lung but none in a piece of the liver. Pancreas unexamined.

Exp. 3. Animal B of Exp. 1 was killed at 9 a. m. on July 19, forty hours after feeding. Two living larvae were found in the abdominal cavity and three in the lung which was slightly blooded, also three in a piece of the liver and one in the pancreas.

Exp. 4. At 11 a. m. on July 19, a guinea-pig was fed with the Strongyloides larvae cultured eight days and at 11 a. m. on the twenty-third, ninety-six hours later, it was killed. Abdominal examination was interfered with on account of bleeding by cutting the liver carelessly. Only one specimen in the pleural cavity, three in the right lung and two in a piece of the liver. Pancreas unexamined.

Exp. 5. At 11 a. m. on July 19, a guinea-pig was fed with the Ancylostoma larvae cultured eight days and at 1 p. m. on the next day it was killed, twenty-six hours after feeding. Two worms were found in the pleural cavity, five in the abdominal cavity, many in the lungs, a few in the liver and three in the pancreas.

Exp. 6. At 9 a. m. on July 24, a guinea-pig was fed with the Ancylostoma larvae and at 3 p. m. on the next day it was killed, thirty hours later. Three were in the pleural cavity, four in the abdominal, many in the lungs, two in a piece of the liver and one in the pancreas.

Exp. 7. During three hours from 9 to 12 a. m. on July 22, Strongyloides larvae six days old were smeared from time to time on the abdominal skin, where the hair was cut off closely and shaved, and the animal was fixed on the holder until 5 p. m. Then it was put in the cage after the smeared part had been cleaned. At 9 a. m. on the next day, about twenty-four hours later, it was killed. Four were found in the abdominal cavity and two in the pancreas, but none in the lungs, pleural cavity or in one-half of the liver.

From the above experiments one may easily assume the two facts that: 1, Ancylostoma and Strongyloides larvae introduced into the alimentary canal of the feeding animal may appear in the abdominal and pleural cavities at least twenty-four hours later, and may penetrate into the liver, pancreas and lungs; 2, Strongyloides larvae smeared on the shaved skin of the abdomen may pierce through the abdominal wall and appear in the abdominal cavity or penetrate into the organs of the cavity in about twenty-four hours.

The piercing power of Ancylostoma and Strongyloides larvae is commonly recognized without which skin infection or further migration into the intestinal wall by the larvae infected, is not easily explained. The above experiments also show the fact clearly that the larvae may pierce through the skin or the wall of the alimentary tract.

Appearance in the pleural cavity of larvae introduced into the alimentary canal is explained in two possible ways: 1, the larvae may pierce through the esophageal wall and reach the pleural cavity and 2, the larvae may pierce through the gastral or intestinal wall to enter first the abdominal cavity, and thence proceed to the pleural cavity by passing through the diaphragm. Previous authors fre-

quently reported finding larvae in the esophageal wall. So the first way may be supposed to exist. From the result of the above experiments as well as from my conclusion obtained by experiments on the migration of ascarid larvae, it seems to me, however, that the second way is the more common and usual course of the larvae reaching the pleural cavity.

Thus I am strongly inclined to believe in the piercing power of larvae in their migration, during which they enter the abdominal cavity by boring the alimentary wall and thence proceed to the pleural cavity by passing through the diaphragm and lastly penetrate the lungs from the surface, as in the case of ascarid larvae. This will be probably a new course for the migration of Ancylostoma and Strongyloides larvae in the body of the host.

Fülleborn described finding Strongyloides larvae not only in the lungs but in the liver and kidneys of a dog fed with the larvae and explained the appearance of the larvae by their migration by way of blood vessels. His theory may be true. However, I believe it is also reasonable to explain the presencec of larvae in these organs by direct penetration from the surface by means of their own piercing power. Some larvae in these organs may certainly be considered to have penetrated from the abdominal cavity which they reached from the intestine by passing through its wall. The larvae in the pancreas of my case may also be understood to have entered the organ from its surface, not by way of blood vessels.

There may be hard places for the larvae to pass from the intestine to the kidney, if they go by means of blood circulation. Thus it will be harder for the larvae to reach the kidneys and pancreas from the intestine by way of blood vessels than by the direct penetration into the organs from their surface by their own piercing power.

Several authors report cases in which they found many larvae in the tissues of the intestinal wall instead of in the blood vessels. Might this not be a case showing their penetration through the tissues? And some investigators described relatively scant occurrence or even absence of larvae in the liver of the infected animal whereas the lungs were heavily invaded. This may, of course, be partly attributed to an incomplete examination, but is also easily explained by assuming the direct migration of the larvae.

I am inclined to believe that the direct penetrating migration of Ancylostoma and Strongyloides larvae in passing through the tissues of the host, is a new course, additional to the old well known route by the blood vessels.

A METHOD OF CONCENTRATION OF PARASITIC EGGS IN FECES

William H. Gates

The microscopic examination of feces for eggs of intestinal, hepatic and some other parasites depends largely for success upon discarding unnecessary fecal matter and concentrating eggs into as small a space as possible. Many methods have been devised (see Hall, Bull. 135, U. S. Dept. Agric.). A modification used by the author is as follows:

After straining through a seive a large quantity of material, or using a smaller quantity without this, feces are centrifuged first with water to wash off surplus lighter material, and later with sodium chlorid, or better, calcium chlorid solution, sp. gr. 1.250, to remove the bulk of the material and float the eggs practically free from sediment. The top one or two cubic centimeters are then removed with a pipette, drawing chiefly from the rim of the meniscus, and centrifuged again with water, which throws the eggs to the bottom. The water is then poured off, leaving *all* of the sediment in the bottom. This sediment is agitated vigorously by holding the tube in the closed hand and pounding on the table. This stirs up all or nearly all of the eggs which may have stuck to the bottom, though a few eggs cannot be removed except with a brush. The sediment is quickly poured into a small dish. The centrifuge tube is rinsed out by squirting water forcibly into it and this also is poured immediately into the dish. The eggs settle rather rapidly and are loosened from the bottom by forcing a little water around the edge to produce a slight whorl. Then before the eggs have a chance to settle, agitate the dish in the same circular direction so that the water tends to form a vortex, gradually diminishing the motion until it is hardly more than a jar. Practically all of the eggs will be found to have settled within a very small field.

For gross examination with the low powers, the eggs may be left in the dish and examined directly. To examine more carefully, under a high degree of concentration, draw up with a pipette a small quantity of water from the center of the mass of eggs; hold this vertical and steady for a half minute or so. Most of the eggs will settle, so that a single drop forced out on the slide will contain nearly all, if not all, of the eggs drawn up into the pipette. For still further concentration, allow the eggs on the slide to settle, and then with a blotter or lens paper very carefully remove a portion of the water from the top of the drop and add another drop. If repeated with care, a large mass of eggs may be collected in the space of a cover slip. This is especially satisfactory if the eggs have been in alcohol for the alcohol will evaporate, leaving the eggs in the center.

BOOK REVIEW

Manual of Tropical Medicine. By Aldo Castellani, C.M.G., M.D., M.R.C.P. and Albert J. Chalmers, M.D., F.R.C.S., D.P.H. Third Edition, New York, 1920, William Wood and Company. 2436 pages, 909 text figures and 16 colored plates.

The appearance of this magnificent volume was so closely coincident with the news of the sudden and unfortunate death of the junior author that the work stands in a very real sense as a monument to his ability and industry, in every way worthy of a career which, though brief, was one of marked achievement.

While the second edition of this manual has been out of print for some years, there has been a natural delay in the preparation of the new edition due to the war and its consequent difficulties in many directions. Despite these, the authors have succeeded in producing a work that is in every way worthy of high praise. So far as mere size goes, the new material introduced has expanded the volume fully one half and the illustrations by an equal amount. Nor has this been all, since the use of smaller type for historical and subsidiary items has allowed the introduction of still more new matter. The work is not only admirably comprehensive without being diffuse but the index, which covers 152 pages, and is in every way well constructed, makes the book useful for rapid reference as few of its size really are.

Much new matter has been added to chapters on plague, fevers, influenza, cat bite fevers, typhus, etc., and the additions incorporate in large part the most recent studies on the relations of animals to these diseases. Entirely new chapters on war zone fevers, diagnosis of tropical fevers, tropical poisonings, myiases and allied conditions, among others, give evidence of the careful efforts exerted to bring the work fully up to date and to enhance its practical value for the worker in the tropics. The rather scanty treatment of tsutsugamushi disease, so important in Japan, is no doubt due to the inaccessibility of the literature, some of the most significant of which is also of very recent date.

Part I of this work is introductory and contains chapters on the history of tropical medicine, tropical races, climatology, foods, diseases, fitness for tropical life. Part II, the classification of diseases in the tropics, has separate sections covering physical causes, chemical causes and parasites, both animal and vegetable. Part III, the diseases of the tropics, has sections on fevers, general diseases, and systemic diseases. Each of these sections is sub-divided on the basis of causation. It is interesting to note that under the fevers thirteen chapters are devoted to those probably of protozoal origin and carried by animals, three chapters to those of bacterial origin, of which two are related to animal carriers, and only four chapters to other types of fevers. In the section on general diseases, animal causation is held responsible for two-thirds of the diseases listed. No better evidence could be given of the tremendous rôle played by animal parasites in tropical diseases. And no one can examine a work like this without being profoundly convinced of the importance of thorough study of animal life for the worker in tropical medicine.

Attention may properly be confined here to those details that fall within the scope of parasitology, although other parts of the book are not only of great interest to workers in medical zoology but are full of valuable material bearing directly upon their subject.

The changes in section C, Parasites, which embraces 740 pages, are so great that one may properly regard this part as a new monograph on the topic. Especially worthy of note is the introduction of a new chapter entitled

The Animal Carriers of Disease, which discusses in thoro fashion the problems connected with animal carriers, relation of hosts, the question of disease reservoirs, contrasts between different types of insect borne diseases, complex life histories of worm parasites and the contrasting relations of bacterial diseases to animal carriers.

Under Animal Parasites the forms are classified in accordance with the zoological system. This has been carefully applied by an author who is not only well acquainted with the animals, but is familiar with the necessary restrictions that have grown up in the subject and that must apply to those who, tho not zoologists, find it necessary to utilize technical material in the field. It would be a piece of good fortune for zoological research and for the proper treatment of parasitic diseases if every worker in this field were compelled to study carefully the material presented in this section of the volume. The authors have made a good move towards the substitution of uniformity for the chaos that exists in medical literature by following, for the names of parasites, as in previous editions, the rules of the International Zoological Congress. It would be hard to find a clearer and more convincing statement of the case concerning nomenclature of parasites than that given in a half page in the preliminary paragraphs under the division on animal parasites.

Undoubtedly the most difficult phase of this topic is that which treats of protozoal parasites. The organisms are so minute, the differences so difficult to determine, and many types are to the untrained observer so thoroly identical, that even in more technical publications there is great confusion with reference to the number of forms that may rightly be distinguished as independent types of organisms. It is a little unfortunate that the author could not have had for consideration the recent comprehensive study of the Amoeba Living in Man by Dobell, reviewed in a recent number of this JOURNAL. The differences between the two accounts are sufficient to be the source of serious confusion to those who have not made an intensive study of the field. Specialists recognize, however, that such differences are inevitable in early work on any topic, and it is not too much to say that we have only just begun to get a grasp on these forms.

There is no synopsis of the protozoa parasite in man in which the topic is presented so completely and at the same time so concisely as in the volume under revision. Its fairness in presenting various aspects of disputed questions and its completeness make the record invaluable for those who are trying to work on these little-known organisms. Chalmers includes the Spirochaetes along side of the Trypanosomes under the Binucleata and thus conforms to the opinion of most zoologists, altho he departs from the views of many pathologists and especially of the bacteriologists who would include them with the bacteria. While it is undoubtedly too early to reach a final conclusion on so complex and obscure a question, yet for the purposes of this volume and of work in tropical medicine the setting given these forms is abundantly justified.

It is indeed in the group of flagellates that the greatest confusion obtains at the present moment. Not only do these forms lack in recognizable morphological characteristics but they are so minute and include so many types that are merely developmental stages that even the trained observer is inclined at times to abandon the attempt to interpret forms he finds. In this field Chalmers has himself worked so long and successfully that the scientific world is fortunate in having him to guide its progress thru the intricate mazes of the problem. And many new genera make their appearance for the first time in these pages in proper systematic relations.

Much new material has been included in the section on the Sporozoa and the work gives the best available survey of this which is another little known field. The treatment of parasitic worms may also be commended for its com-

pleteness and for the judgment exercised in introducing new material. One finds little to criticize; perhaps the accounts of the life history of Ascaris and of Schistosomes are the most imperfect.

The short chapter on the leeches is rather unsatisfactory and the single figure given is really little more than a joke, but this group is no doubt of all the least significant for the worker on tropical diseases. The Arthropod chapter is well handled, if one excuse the omission on grounds difficult to suggest in view of the completeness of the work otherwise, of the splendid work done by Howard and his colleagues on mosquitoes.

Some minor points deserve passing criticism; the authors use, for instance, in a synopsis the names Platyhelmia and Nemathelmia which appear in other places as Platyhelminthes and Nemathelminthes. Such variant forms, easily recognized by the specialist, are apt to be serious stumbling blocks for those not trained in zoological lines. Tho some of the misprints of the earlier edition have been corrected, others still remain not merely in the names of well-known scientific workers and journals, but in a few scientific names where it might be difficult to recognize in Collyrichum and Hocyalomma the correct forms Collyriclum and Hyalomma. It is unfortunate to find such terms as *Rattenkönig cercariae,* which is not even a correct citation of the German term and is readily translatable by the equivalent Gorgonocephaloid.

On the whole the figures are admirable and abundant. The colored plates are very well done and the photographs of eggs by Bell are both new and thoroly desirable, altho some of them have come out in the printing rather indistinctly and the value of the representations (p. 626) of such as are of doubtful identification is seriously reduced in that no indication is given of the magnification or actual size. The literature lists have unfortunately not been revised and stand almost everywhere exactly as in the earlier edition, altho much new and very important material has been added to the text. This throws a heavy burden upon the student who wishes to refer to the recent contributions in order to follow up more fully an individual problem. Typographical errors seem to be more frequent in authors' names and in the designation of periodicals than elsewhere, altho it must be confessed that the percentage of such errors is fortunately small.

It would be hard to record the excellencies of the work with the same fulness with which the minor errors have just been reviewed. From cover to cover the volume shows adequate knowledge and control of the field. Its conciseness and clearness of presentation cannot be too highly commended. The fulness with which it includes most recent work, and the freedom from bias in dealing with moot questions and unsolved problems are admirable features that must be emphasized in closing this review. The work is a storehouse of splendidly arranged material, and as such is indispensable to every worker interested in any phase of medical zoology.

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ETIOLOGY OF TSUTSUGAMUSHI DISEASE *

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An endemic infectious disease, called Tsutsugamushi disease (Kedani fever, river fever), occurs in Japan along the rivers in the northern provinces. In spite of the persistent efforts of numerous workers, its etiology has been very difficult to determine. This peculiar disease has a close resemblance to Rocky Mountain spotted fever in America, but it is transmitted by a different insect. The carrier of the Japanese disease is a minute red mite, abundant in the infested region, that is, in the low lying cultivated fields along the Shinano river, between the riverside and embankments which vary up to one and a half miles from the river. These fields are at times during the summer covered by overflow water. In nature the mite is ectoparasitic in the ear of field mice, but it freely attacks human beings as well as other animals whenever accessible. Zoölogically, it is considered *Leptus akamushi* (Brumpt), a species distinct from the well known *L. autumnalis* of Europe.

The early clinical course of the disease is marked by a gradual rise of the body temperature, a characteristic lesion at the site of the bite, and swelling of adjacent lymph nodes. The incubation period is about eight days. The fever, which is usually 38°-39° C. in the early stage, may rise to 40° C. or even higher, and usually lasts three or four weeks. Complete recovery requires a month or more. In bad cases a fatal result follows about ten days after the onset. The mortality of the disease is approximately forty per cent.

* My work on this disease was started in 1906 and has been continued up to 1919. Many of my findings in the course of these twelve years' investigation have been published in Japanese medical journals (Hokuetsu Igakkai Zasshi, no. 156, 1906; no. 158, 1907; no. 162, 1908; no. 165, 1909; no. 168, 1909; no. 173, 1910. Chu-o Igakkai Zasshi, no. 124, 1915; no. 127, 1916; no. 130, 1916. Hayashi, N., Mukoyama, T., Oshima, F.: Chu-o Igakkai Zasshi, no. 138, 1918; 1919 [XXVI] no. 2). A summary of these findings together with new facts and conclusions with regard to the etiology of this disease is of especial interest in connection with recent American contributions to the study of Rocky Mountain spotted fever. I have been very much pleased to find in Dr. Wolbach's recent detailed description of the organism found in the American fever, a very close similarity to the bodies which I found in the closely related Japanese disease.

The microscopical examination of fresh material in the hanging drop shows a number of refractile, spherical bodies, each measuring from 1 to 3μ in diameter, without any perceptible movement. They rarely occur in clusters, but two or three of them may often be seen linked together. As a rule, when two bodies are coupled, one is decidedly larger than the other. The smaller half of the couple often appears as if it were a pseudopodium of the larger. I discovered these peculiar bodies in 1906, and designated them as the Spheroid Bodies. The examination of blood shows, in addition to these, others which I have called the "rod-bodies." There are two kinds, the first large and the second very small; the large one measures about 7 by 3μ. It contains a spherical refractile area resembling a nucleus and a number of much more refractile granules toward the periphery, and is always found close to the surface of the red blood cells. The small "rod body" measures about 3 by 0.5μ and resembles a bacterium. It shows no evidence of motility.

Varying numbers of the spheroid as well as rod bodies are often found embedded in the cytoplasm of the cells of the exudate and of lymph nodes. Fresh sections examined in normal salt solution, glycerine or dilute acetic acid show these bodies intracellularly, and their morphological characters are identical with those that are found extracellularly.

The lesion which develops at the location of the bite is quite unique, being comparable only with the chancre of syphilis. In the earliest stage that can be identified, the wound is much raised and is about the size of a small pea, being 2-3 mm. in diameter. It has first a reddish purple color, and later a blackish necrotic appearance. A smear from the wound at this stage shows a large number of very small rod and spheroid bodies, but cultures taken from the same source at the same time are entirely negative. The wound usually commences to heal through the regeneration of surrounding tissues when the case is on the way to recovery, and thus shows close parallelism with the general clinical course of the disease. Obvious as is the importance of the study of the wound, it has received little attention. On account of its being exposed, thus offering opportunity for various secondary infections, the result of observations are of little value unless controlled by bacterial cultures in every case.

At the onset of the fever, the minute bodies appear in great abundance in the smear. Most of them are seen to be more or less enlarged. Even at this stage cultures do not demonstrate any bacterial infection. At the height of the fever, when the superficial, degenerated tissue of the lesions falls off, leaving a small ulcer, there usually occurs a secondary infection with common bacteria such as staphylococci, as

detected by cultures. In mild cases, the rod and spheroid bodies, as well as bacteria, gradually decrease in number, hand in hand with the decline in fever.

In later stages, with necrosis and ulceration of the lesion, the exudate usually contains bacteria. The wandering cells which appear in large number in the lesion are actively phagocytic. Some of these phagocytes contain in addition to degenerative products of red blood cells and tissue cells, a large number of rod as well as spheroid bodies. The manner in which the minute bodies are included in cytoplasm of these cells is identical with the conditions in the cells of lymph nodes.

The rod-bodies are extremely small, measuring 1-2, rarely 3μ in length. Loeffler's methylene blue and ordinary Giemsa solution stain them blue throughout like bacteria, but Prowazek's trachoma granule stain brings out in the rod-body, a circular area of chromatin substance staining purplish, leaving the rest of the body blue although not as distinct as in protozoa. Hematoxylin also gives a staining reaction of the circular area characteristic of chromatin. The rod-body is typically more or less pointed at one end, and slightly rounded at the other; the chromatin area being at the round end (Pl. X, Figs. 18, 24). Some of the rod-bodies show a chromatin area at each end (Figs. 19, 20, 21). In those that are as long as 3μ the chromatin substance may appear to lie outside the body and attached to one end of the latter by a narrow belt of achromatic substance. (Pl. X, figs. 25, 26; Pl. XI, figs. 3, 5). The largest of the rod-bodies may measure 7 by 2μ and may even at a glance appear like trypanosomes (Figs. 15, 16). These giant forms also contain either at the end or middle, one or two masses of chromatic substance.

These spheroid bodies are comparatively large, being $2\text{-}3\mu$ in diameter, and stain deeply like cocci. A characteristic figure often seen shows two spheroid bodies of distinctly different sizes coupled together (Pl. X, Figs. 27, 28, 31), or in a manner resembling diplococci (Fig. 29, 30, 6 B), or even in chain formation. Frequently chromatic substance in the center stains more deeply than the peripheral area. These characteristic features are more conspicuous in larger forms than in smaller ones.

The swelling of lymph nodes adjacent to the bite takes place in the early clinical stage, when the wound is still elevated and the body temperature only slightly raised. Although some lymph nodes were removed by operation in an early stage the greater part of my material was obtained after the development of distinct fever (38.5°-39° C.). All of the lymph nodes in the region manifest swelling though in different degree. At the height of the fever, material was often taken by puncture with the syringe; it was collected at autopsy, and also by operation at various stages in experimental infection of animals.

Examination of sections and smear preparations has shown the presence of peculiar granules, which are taken up by the giant phagocytes of endothelial nature. These granules and phagocytes occur most abundantly around the bite in the adjacent lymph nodes and spleen. Morphologically, three types of granules and phagocytes are found in the cytoplasm of the large mononuclear lymphoid cell, namely:

(a) A large ring-shaped chromatic body with an achromatic area (Pl. I, 1a; Pl. XI, Figs. 10a, 11a, 12a, 13a).

(b) A spherical body showing deep and uniform blue staining, but containing reddish chromatin substance having a bipolar distribution, and resembling a diplococcus (Pl. IX, Fig. 1B, 1b).

(c) An exceedingly small rod shaped body, often more of comma-shape, or with dumb-bell-like construction. This third type of the granules is most abundant (Pl. IX, Figs. 2, 3, 4, 5).

In 1915, I found granules identical with those just described in small lymphocytes from lymph nodes removed early by operation. Examined with the Giemsa-Prowazek stain, minute granules can be demonstrated in the narrow area of blue cytoplasm around the purple nucleus. With the aid of a Zeiss $\frac{1}{12}$ objective and ocular 6 or 8, the granules can be easily classified into the three types already described. Here, more than in other cases, the rod-shaped type predominated. The rod-body is more or less rounded at one end, and pointed at the other. A proper stain shows the round end reddish purple, and the pointed end blue. Delafield's hematoxylin brings out similarly the three types of granules, and also differentiates clearly the round end of the rod-body.

In general, the granules are fairly constant in size. The number embedded in a single cell varies from a few to approximately two hundred, but more commonly from ten to sixty. These are usually more or less localized in the enlarged portion of the cytoplasm (Pl. IX, Fig. 2; Pl. XI, Figs. 2a, 3a), or in the area of the cytoplasm formed by the indented nucleus.

The lymph (from lymph nodes) also contains three types of the granules. Here, too, the granules may be found in clusters in many cases, although some may be solitary. In addition, the fluid often contains a fourth type of granule, round in shape with a centrally located red-staining spot and a narrow achromatic ring, which is in turn surrounded by a finely granular peripheral zone (Pl. XI, Figs. 17, 18, 19). I have given the name of "oocystoid body" to this type.

It is natural to suppose that the virus of Tsutsugamushi disease may be found in the blood, after it enters the body of the patient through the bite. Its demonstration would be of great value, especially as affording a comparatively simple means of accurate diagnosis. Cover

glass smears from a large number of severe cases all showed the presence of the peculiar granular bodies, in or on the red corpuscles. In mild cases, or those in very early or late stages, none, or a very small number of the bodies were observed. These bodies may also occur in any type of white blood cell, and, moreover, may be seen scattered freely in the blood plasma. These facts I reported in 1906.

The granular bodies are minute, strongly refractile, and usually found within or on red corpuscles (Pl. X, Figs. 6c, 7). A highly magnified picture of Giemsa-Prowazek stained specimens (Pl. X, Figs. 6, 7) shows that these bodies are in no way different, as far as could be observed, from the minute bodies already described in the lymphoid cells. They are usually comma-shaped, but often modified into rod or dumb-bell shapes. They are, as a rule, evenly distributed within the cell, although sometimes grouped in one part of it. The number of the bodies in a red cell varies from a few to many. Enlarged forms, such as are found in the cells of the lymph nodes, also occur in the blood (Pl. X, Fig. 6A, 14). The minute bodies may be seen free in the blood plasma during the eruptive stage even in the case of a slight attack. The smaller forms of these extra-cellular bodies may appear in a thickly crowded group (Pl. XI, Fig. 1, 16a), while some of the larger forms may be found coupled with smaller ones (Pl. X, Figs. 15, 16, 17). This phenomenon of coupling among the larger bodies is of special interest, as it takes place only in very severe cases.

Sometimes with the Giemsa-Prowazek stain a chromatin mass surrounded by an achromatic ring was seen at one end of the body (Pl. X, Figs. 12, 13, 14). Some bodies are round (Figs. 12 13), others elongated (Fig. 14). The former is like a ring body of the malaria plasmodium; the latter has two (end and center) chromatin spots, the central being very small. Delafield's hematoxylin also brings out these chromatin spots.

The enlargement of the spleen in Tsutsugamushi disease, usually encountered at autopsy, can be easily detected clinically. Material taken by puncture with a syringe always contains a large quantity of red cells, but pulp-cells are rarely seen. The minute granular bodies are abundantly demonstrated in these cells in many cases. This is also true in the case of autopsy material. Generally speaking, the smaller type of granular bodies seems to predominate in this organ (Pl. XI, Fig. 8a).

ANIMAL EXPERIMENTS

1. Infection through the bite of the mite: Animals (monkeys, rabbits, guinea-pigs, rats and calves) were allowed to be attacked by the mites in the infested region, and after recognizing the evidence of the bite on them, development of symptoms was carefully watched for.

The monkey shows the most typical rise of body temperature, the guinea-pig a less typical fever, and the calf only a slight rise of temperature. The rabbit shows no change.

Positive infection of a monkey (*Pithecus fuscatus* Blyth, the short-tailed species occurring in Thikoku, Japan) through the bite of the mite was first proved by the author in 1906. In this animal, the typical lesion of the bite developed, along with the enlargement of the lymph nodes and the characteristic fever, four or five days after the bite was recognized. The general clinical changes closely resemble those in human cases, even to the occurrence of leukopenia. The characteristic minute bodies, described from human cases, are present in the phagocytic cells of lymph nodes and spleen; in a later stage, also in those of bone marrow, and still later in most of the other internal organs. These bodies were also found free in body fluids. The infected monkeys usually recover in a week or two.

Guinea-pigs show no special pathologic change at the site of the bite, although swelling of adjacent lymph nodes and fever develop within several days after they are bitten. In these cases, too, the minute bodies of several types were abundantly demonstrated in the lymph nodes, blood and other tissues.

2. Transmission Through Injection of Infected Blood: The typical symptoms of the disease were successfully reproduced in the monkey by subcutaneous injections of the blood from the severe human cases. The diseases can also be transmitted from the monkey thus infected to other monkeys by inoculating them with blood drawn from the former. Similar experiments with guinea-pigs also gave positive results. Moreover, the injection of blood or emulsified organs from infected guinea-pigs into other animals, into monkeys, was followed by the development of unmistakable symptoms.

3. The Relation of the Mite and Field Mice: As stated already, the mite is parasitic on the inside of the external ear of the field mouse, which is very abundant in the infested region. In 1910, I was able to demonstrate that the field mouse is a bearer of the virus of Tsutsugamushi disease.

Aerobic and anaerobic cultures, on various solid as well as liquid media, were repeatedly taken from a large number of human and animal cases, always with negative results. A few of the common varieties of bacteria or often yeasts developed in some cultures, but no organism that can be considered as the causative agent of the disease has ever been detected in any of them.

By using Kleine's (1905) piroplasm medium, it was possible to recognize the presence of the minute bodies described in this paper, but no definite growth has been demonstrated on this or on the Novy-Mac-

Neal-Nicoll medium. The cocci bodies, grown on Loeffler's serum-agar by Nagayo and his associates in 1917, could not be found in repeated experiments which I made with the same medium. The possibility of cultivating the virus of the disease has not thus far been definitely demonstrated.

Under natural conditions healthy individuals do not contract the infection directly from patients suffering from the disease, the virus being transmitted only through the mite. It has been noted in earlier publications that a certain degree of immunity follows recovery from this disease. A second infection may occur some years later but the case is never severe. A third, or even fourth infection, in a very light form is also known. In animals, however, I found that no second infection was possible, in spite of many trials. I have also noted that the first infection is very mild in children or in young individuals, while in adults over forty it is frequently fatal.

The resistance of the virus to thermal change and putrefaction is very slight, as virus from human as well as animal cases is no longer virulent a day or two after it has been taken. Another important fact is that the virus is absent from the filtrate through Pukall or Berkefeld filters. This point, first reported by Kitajima and Miyajima, was recently confirmed by Nagayo.

DISCUSSION

1. The Granular Bodies in the Lymphoid Cells: The larger types of these ring or spheroid bodies are found in large mononuclear cells, as well as in the cells of the spleen pulp, while the minute type (rod bodies) are embedded in the cytoplasm of small mononuclear lymphoid cells.

Since Ehrlich's classical work it has been believed until very recently that the mononuclear lymphoid cell is not granulated under either normal or pathological conditions. Among others, Pappenheim and Schridde have shown the occurrence of fuchsinophile granules, which are now known to be the true mitochondria. More recently azure granules of problematic nature have also been described in this type of cells. These granules are, however, of different chemical nature from my granular bodies, as can be seen from their staining reactions. It is beyond doubt that the former could not be considered identical, or even genetically related to the latter. An extensive examination has failed to demonstrate the granular bodies in lymphoid cells in normal individuals, leading to the conclusion that they are not normal constituents of these cells. Nor have the granular bodies been observed in the lymphoid cells in any case of lymphoid hyperplasia that I have examined.

The impossibility of the granular bodies being any sort of degeneration products, which might become phagocyted by lymphoid cells, is none the less evident in view of my microscopical observations. The morphological characters and the regularity in the modification of these bodies, as detailed early in this paper, preclude any conclusion that they are degenerative debris, and suggest very strongly their being peculiar micro-organisms. For the same reasons the granular bodies must be considered as different from Prowazek's so-called "reaction products" in the infections of various sorts of filtrable virus. The granular bodies in question are absolutely peculiar to Tsutsugamushi disease and must not be overlooked in the study of its etiology.

2. The Granular Bodies in the Large Mononuclear Cell: The large mononuclear cell, called endothelial in my report in 1916, Kiyono terms histiocyte. The occurrence of the granular bodies in this type of cells is a common finding in Tsutsugamushi disease. The granules are either rod-shaped, spherical or ring-shaped, and these three types may be found side by side in a single cell. They are identical in staining reactions but differ in size. It is very probable that the three types represent stages in a series of modifications undergone by the same organism. It seems that the minute rod-shaped body gradually grows, assumes a spherical form and then after further growth the ring shape. The semilunar, or crescent shape often taken by some much enlarged bodies may be considered as due to still further growth. Some of the ring-shaped bodies may also be coupled. It is very interesting to find on close examination that these enlarged bodies contain a number of exceedingly minute granules. If the interpretation suggested proves to be correct, the granular body must be an organism, and more specifically a protozoon, which has intracellular stages in its life cycle.

3. Granular Bodies in the Red Cell: In view of the fact that these bodies resemble the ordinary basophilic granules in the red cells, the distinctive characteristic between them may be pointed out. As first made known by M. Askanazy, Grawitz and others, the basophilic granules of red cells appearing under certain pathologic conditions, are nothing more than ill-defined, minute particles. Contrary to this the "granular bodies" found by me are large enough to be measured. Moreover, they are very clearly differentiated from the protoplasm of the red cells. On destaining the true basic granules become obscure very rapidly, while the "granular bodies" stand out clearly by their peculiar refractility, sharply defined edges, and finally by the characteristic chromatin spots which still retain a reddish purple color.

In short, the "granular bodies" with their organized structures cannot be confused with the true basic granules which have no definite

morphological characteristics. I conclude, therefore, that the "granular bodies" in the red blood cell are of the same nature as those in the lymphoid cells. Since the red cells as well as the lymphoid cells contain the "granular bodies" only in cases of Tsutsugamushi disease, these bodies must be considered seriously in connection with the etiology of the disease.

4. Free Bodies in Plasma: In the foregoing I have stated that the minute bodies usually found embedded in the cytoplasm of lymph cells may also occur free in the lymph, either solitary or in groups, and that these bodies may be rod, spheroid, or ring-shaped. An oocyst-like appearance was also assumed by some of them. Swarms of the minute "comma" or "rod-bodies" often mixed with longer dumb-bell shaped ones, were observed in the plasma in blood smears. Also these bodies have been found in addition to bacteria in serum from the wound at the site of the bite.

The free bodies are not abundant in mild cases but in severe cases their appearance is conspicuous. The free bodies often appear earlier than the intracellular bodies, and may occur in the wound along with ordinary bacteria.

Since the minute bodies in lymphoid cells are peculiar to Tsutsugamushi disease, the free bodies should also be considered of the same significance inasmuch as they have not been found under any other conditions. Morphologically, moreover, a minute comparison fails to detect any perceptible difference between the intracellular and free forms.

In their staining reactions and morphology alone the bodies which I have described often resemble bacteria, especially as the cytoplasm and chromatin substance are differentiated with difficulty, but there is no other evidence of their bacterial nature. In smears from blood and lymph nodes I have found bodies in both red cells and lymphocytes identical in appearance with the ring forms found in the red cells in malaria (Pl. IX, Fig. 1a; Pl. XI, Figs. 4b, 5b), showing a structure never found in bacteria.

I described these minute granular bodies in my first report on the virus of Tsutsugamushi disease and from various findings concluded that they represent a species of protozoa. It was suggested that the organism might be either Piroplasma or some related form. In a further paper I classified the organism as Piroplasma, referring to *Theileria parva* of African cattle fever, and tropical pirosomes in cattle described by Dschunkowsky and Ruhs, as most closely related forms.

In order to indicate the basis of my conclusion, the following facts should be emphasized :

1. Tsutsugamushi disease is transmitted by certain species of mite; the original bearer of virus in the infected region is the field mouse on which the mite is parasitic.

2. Tsutsugamushi disease is characterized clinically by the lesion, high fever, and swelling of lymph nodes. Decrease in the number of leukocytes is well marked in the blood picture. There is also a gradual decrease in red cells, but it is not conspicuous.

3. Anatomically the lesion is characteristic, also the swelling of lymph glands and splenic enlargement; the blood is less coagulable. Internal organs undergo parenchymatous degeneration and often show small areas of necrosis and thrombi.

4. Experimentally it is possible to transmit the infection with typical symptoms by inoculation of fresh blood or other tissues, from human as well as animal cases experimentally.

5. No bacteria which can be considered as the cause of the disease have been cultivated on media, either from clinical or experimental material, nor have any spirochaetes been found.

6. Under normal conditions the virus is not transmitted directly from man to man. It is least resistant to dryness and heat, and unfilterable.

With the above facts on one hand, and on the other the knowledge that the rod, spheroid and ring-shaped bodies, occur in all internal organs and in the blood and especially because of the early appearance of the rod-bodies in lymph cells adjacent to the bite, I have reached the conclusion that the virus of the disease is the species of Piroplasma in question. Among various species of Piroplasma, the cause of African cattle fever, *Thieleria parva,* (see Gonder, 1911), seems closely allied to the forms found in Tsutsugamushi disease, on account of morphological similarity, and of affinities for lymphocytes or endothelial cells.

In the case of *Theileria parva,* however, it is impossible to infect normal cattle by subcutaneous injection of diseased blood, while in Tsutsugamushi disease such a transmission is easily secured through an injection of a very small quantity of infected blood. In this respect the tropical pirosomes, described by Dschunkowsky and Ruhs (1904) from the Caucasus, shows a closer approach to my organism since in the former infection can be transmitted mechanically, as in the latter. In addition the tropical pirosome agrees with my granular bodies of Tsutsugamushi disease in having an intracellular stage and also three different forms, rod, spheroid and ring bodies.

The tropical pirosome deviates from the organism found in Tsutsugamushi disease in its typical protozoon staining reaction, and also in

that it does not infect man and cannot be transmitted experimentally to monkeys. The blood parasite of the mole, *Grahamella protista,* Wolbach's (1914) organism in Rocky Mountain tick fever, and *Bartonia bacilliformis* described by Strong and others (1915) in oroya fever, are also more or less closely related but are undoubtedly specifically distinct. I consider the organism in Tsutsugamushi disease as a hitherto undescribed species, and at the suggestion of Dr. Henry B. Ward designate it as *Theileria tsutsugamushi,* n. sp. I am inclined to believe that further study will justify the inclusion of this species in a new genus clearly distinct from that in which it is placed here.

THE PROBABLE LIFE CYCLE OF THE ORGANISM

Since it has not been possible to cultivate the organism, the following successive changes in preparations is the only method for studying its life cycle. For this purpose, all the varieties of granular bodies I have observed were faithfully sketched from preparations (Plate XI):

(a) In Lymphoid Cells: The granular bodies in their smallest form (rod-shaped) are at first chiefly localized in one part of the cytoplasm (Figs. 2a, 3a, 4a). As they increase in size, they become more evenly distributed in the cell body (Figs. 5a, 6a, 7a, 8a) and some show dumb-bell, spheroid, or even ring-shapes. Cells containing large bodies, (Figs. 9a, 10a, 11a, 12a, 13a) gradually disintegrate, setting these bodies free (Figs. 14a, 15a, 16a). At the same time, the chromatin portion of the larger body becomes granular in structure and finally breaks up into minute comma-shaped granules (Fig. 1).

(b) In Red Cells: The changes are similar. A group of minute granular bodies (Figs. 1b) gradually breaks up as they grow into enlarged rods (Figs. 2b, 3b) which continue to increase in size (Fig. 4b) until they become ring-shaped and finally dissociate themselves into minute granular bodies.

(c) In the Plasma: Arranging the free granular bodies according to sizes one would naturally take the minute granular form (Fig. 1) as the starting point, successively followed by the comma or rod form (Figs. 2, 3) and by the spheroid (Figs. 4, 5, 6, 7, 10) or ring-shaped, malaria-like form (Figs. 4 to 9). They may then assume an amoeboid appearance (Figs. 11 to 17) and elaborate minute granules within themselves. These may early break up into minute comma-shaped bodies (Fig. 1) or may form Koch's free "Plasmakugeln" (Figs. 19 to 22) and finally dissolve into comma or rod bodies (Fig. 2).

It is worth noting that in the above interpretation, the stages in the life cycle of my organism correspond very closely to those in the life cycle of *Theileria parva,* as worked out by Gonder.

From the above data the life cycle of the organism of Tsutsugamushi disease may be constructed as follows:

The rod, spheroid or ring-shaped bodies found intracellularly in lymphoid and red blood cells, represent an agamic generation. In progamic and gametic generations the organism is free in the blood plasma, assuming rod or ring shapes (Figs. 2-9), but forms shown in figures 4′, 5′, 6′, 7′, 10 are agamic. In the gametic generation it is transformed into an ameboid body (Figs. 11-17). During the metagamic generation the ameboid body comes to assume an oocyst-like appearance on account of the development of numerous small granules within it (Figs. 16-18). The oocystoid body then breaks down and sets free the granular inclusions, namely the metagametes. The metagametes (sporozoits) are the smallest units of the organism.

Theileria parva is found in the intestine or in the salivary gland of the tick during the metagamic generation. In my organism, however, this generation is also seen in the blood of the patient. In Tsutsugamushi disease, therefore, the mite may not be the necessary intermediate host. In this connection the possibility of infection with this disease by injection of infected blood is of much interest.

It is known that pirosomes multiply by fission. Arguing from analogy then, it is probable that the organism of Tsutsugamushi disease may also, under certain conditions, reproduce in a similar manner. As a matter of fact this organism often assumes a dumb-bell shape suggesting transverse division. If this crosswise division is really a process of reproduction, it should be counted as one of the peculiarities of this organism. The possibility undoubtedly exists that the larger type of dumb-bell shaped bodies appearing in the gametic generation may represent copulation of male and female elements (Pl. XI, Figs. 14, 15, 16, 17), instead of division. It is not possible to come to a definite conclusion on this point at present. The organism of Tsutsugamushi disease differs from Gonder's *T. parva* by its minute comma-shaped true sporozoit.

SUMMARY

1. Minute bodies, described as "rod," "spheroid" and "ring-shaped," have been found in the lymphocytes of lymph nodes and in mononuclear endothelial phagocytes of the spleen and lymph nodes, and in the region of the bite in patients suffering from Tsutsugamushi disease. They also occur free in the blood plasma, and in severe cases in the red cells.

2. The disease has been transmitted experimentally to monkeys, guinea-pigs, rabbits and calves. Bodies similar in appearance and distribution to those found in human cases, have been demonstrated in experimentally infected animals.

3. These bodies, on account of the difficulty of differentiating their cytoplasmic and chromatic elements, resemble bacteria, but no evidence of their bacterial nature has been obtained from cultural or animal experiments.

4. Cultural experiments have proved wholly negative.

5. Microscopically, these bodies are found to possess definite morphology, with parts comparable to the nucleus and cytoplasm of a cell. Moreover, the three principal types in which they appear (rod, spheroid and ring shapes), merge into each other. From these facts, it is concluded that the organism in question is a protozoon. Bodies of different sizes represent different stages in its life cycle.

6. Biologically, this protozoon is to be considered as a new species, resembling but showing differences from *Bartonella bacilliformis* and *Theileria parva*. The parasite to which it appears most closely allied is *Theileria parva*.

7. The organism found in Tsutsugamushi disease, I have designated tentatively as *Theileria tsutsugamushi,* spec. nov.

Here, I wish to express my sincere thanks to Professor A. Fujinami for his continued encouragement and help, and to Mr. Kuhara of Osaka for his generous aid in support of our work. A great deal of credit is also due to the assistants in my laboratory who have participated in the investigations.

References Cited

Dschunkowsky, E., and Ruhs, J. 1904.—Die Piroplasmosen der Rinder. Centr. Bakt., Orig., 35:486.

Gonder, R. 1911.—Die Entwickelung von *Theileria parva,* dem Erreger des Küstenfieber der Rinder in Africa. Arch. f. Protistenk., 22:222.

Graham-Smith, G. S. 1905.—A New Form of Parasite Found in the Red Blood Corpuscles of Moles. Jour. Hyg., 5:453.

Kleine, F. K. 1905.—Ergebnisse der Forschungen R. Koch über Küstenfieber und über die Pferdsterbe gelegentlich seiner letzen Expedition nach Sud-Africa. Deutsche med. Woch., 1:912.

Meyer, K. F. 1913.—Afrikanische Küstenfieber. Handbuch path. Mikroorg. Kolle u. Wassermann, 2 Aufl., 7:538.

Strong, R. P., Tyzzer, E. E., and Sellards, W. A. 1915.—Report of Tropical Expedition in 1913.

Wolbach, S. B. 1914.—The Etiology of Rocky Mountain Spotted Fever. Jour. Med. Res., 36:121.

Explanation of Figures

PLATE IX

Drawn from smear preparations with camera lucida and Zeiss 1/12 apo. obj. and comp. oc. 6. Stained by the Giemsa method (Prowazek's trachoma granule stain).

A. Ring-shaped body. *B*. Spheroid body. *C*. Rod-shaped body. *a*. Small ring, and coupled rod and spheroid bodies. *a'*. Small ring-shaped body showing chromatic spot (resembling malaria ring form). *b*. Small spheroid body. *c*. Small rod shaped body.

Fig. 1.—Minute bodies embedded in large mononuclear endothelial cell.

Fig. 2.—Same in small mononuclear lymphoid cell, showing coupled rod and comma-shaped bodies.

Figs. 3, 4, 5.—Similar to Figure 2.

(The plates which are included here through the courtesy of the author are reprinted from the Japanese originals. There they bore the numbers I, II, and III which to fit the series in The Journal have been changed to IX, X, and XI.—Editor.)

PLATE X

Drawn from a smear preparation taken from patient's blood (First published in 1906, Hokuetsu Igakkai Zasshi, No. 156). Drawn from smear made from patient's blood; magnification and staining as in Plate IX.

Fig. 6.—*A,* large, *a,* small ring-shaped body. *B,* large, *b,* small spheroid body. *C,* large, *c,* small rod-shaped body.

Fig. 7.—Large and small rod-shaped bodies embedded in red blood cell.

Figs. 8, 9.—Large rod body, showing three chromatic spots.

Figs. 10, 11.—Small rod and ring-shaped bodies.

Figs. 12, 13.—Ring bodies resembling malaria-rings.

Fig. 14.—Large rod body.

Figs. 15-17.—Large gametocytes connected together by thin thread.

Figs. 18-31.—Smear from bite wound.

Fig. 18.—Rod bodies (*a*) showing achromatic area around chromatin substance. Also another small body with different morphology.

Figs. 19-21.—Rod bodies showing indistinct chromatin substance.

Figs. 22, 24, 26.—Same as figure 18a.

Fig. 24b.—Rod body with two chromatic spots.

Fig. 23.—Same with three achromatic spots.

Fig. 25.—Body with very striking achromatic area.

Figs. 27, 28, 31.—Two coupled gametocytes.

Figs. 29, 30.—Gametocytes; large spheroid body, same as fig. 6 B.

PLATE XI

Diagram of supposed life cycle of *Theileria tsutsugamushi.*

Figs. 1-22.—Free intercellular stage.

Fig. 1.—Sporozoites.

Fig. 2.—Rod-shaped bodies.

Fig. 3.—Elongated rod bodies.

Fig. 4.—Minute spheroid bodies.

Fig. 5.—Same as figure 3, but with an accessory small chromatic spot.

Fig. 6.—Enlarged spheroid bodies (macrogametocytes).

Figs. 7, 8, 9.—Same, like malaria ring body.

Figs. 4, 5, 6, 7, 10.—Spheroid bodies, resembling diplococci.

Figs. 11, 12.—Ring bodies assuming amoeboid appearance.

Fig. 13.—Same, enlarged form.

Fig. 14.—Large bodies coupled together.

Figs. 15, 16.—Large rod bodies, resembling trypanosomes. Note the two nuclei of different sizes and finely granulated protoplasm.

Fig. 17.—Large spheroid bodies, showing fine granulation (oöcyst formation).

Fig. 18.—Same, showing chromatic spot with achromatic ring.

Fig. 19.—Minute granulated body (Koch's free Plasmakugel).

Figs. 20, 21, 22.—Same, containing large granules.

Figs. 1a-16a.—Intracellular stages passed in lymphoid cells.

Fig. 1a.—Spheroid body embedded in mononuclear cell of lymph gland.

Fig. 2a.—Group of minute comma or rod-shaped bodies in mononuclear cell of blood.

Fig. 3a.—Same, showing grouping in mononuclear cell of lymph gland.

Figs. 4a-5a.—Same, showing less localized condition of the bodies.

Figs. 6a-7a.—Same, showing no localization.

Fig. 8a.—Rod and minute ring bodies in splenic cell.

Fig. 9a.—Same, in small round cell of lymph gland.

Fig. 10a.—Same as Plate IX, figure 1.

Fig. 11a-12a.—Rod, spheroid and ring bodies in large mononuclear cell of lymph gland. Fig. 12a also shows cell containing remnants of two rod cells.

Fig. 13a.—Similar to 11a.

Fig. 14a-15a.—Free large ring-shaped bodies identical with intracellular forms.

Fig. 16a.—Free ring-shaped bodies breaking down into minute granular bodies.

Fig. 1b-5b.—Intracellular stage in red blood cells.

Fig. 1b.—Group of minute comma-shaped bodies in red blood cell.

Fig. 2b.—Same, comma- or rod-shaped bodies slightly enlarged.

Fig. 3b.—Same, showing further enlargement of comma- or rod-shaped bodies and a few large rod-shaped bodies.

Fig. 4b.—Two large rod-shaped bodies adhering to surface of red cell.

Fig. 5b.—Ring-shaped body, and body showing formation of minute granulation.

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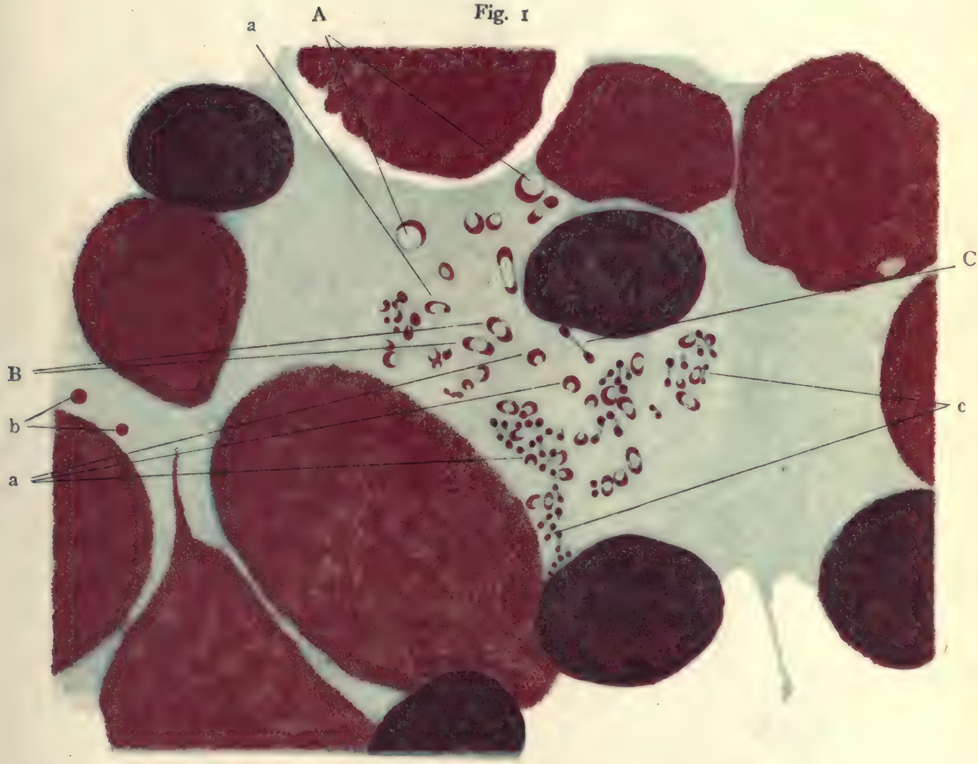
Plate I

Fig. 1



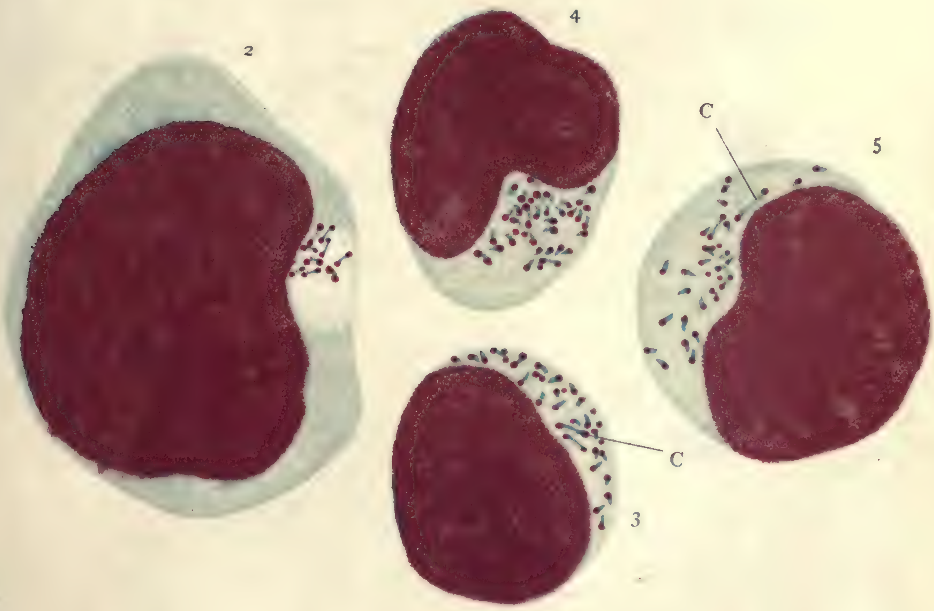


PLATE IX

Plate II

Fig. 6

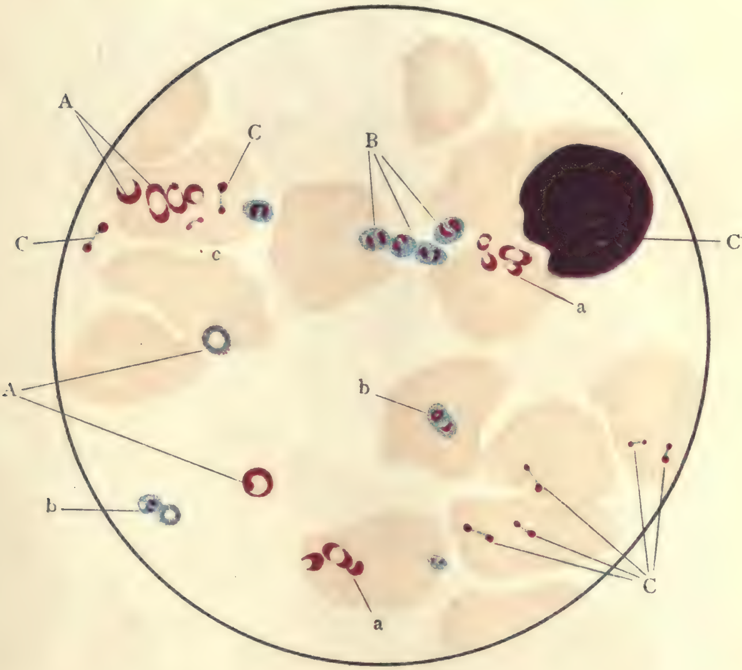


Fig. 7

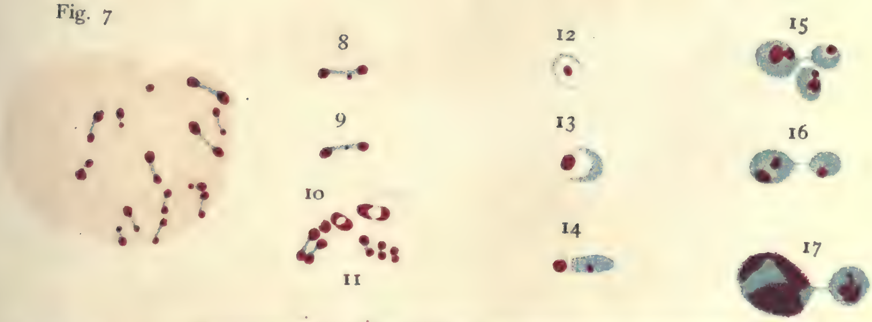


Fig. 18

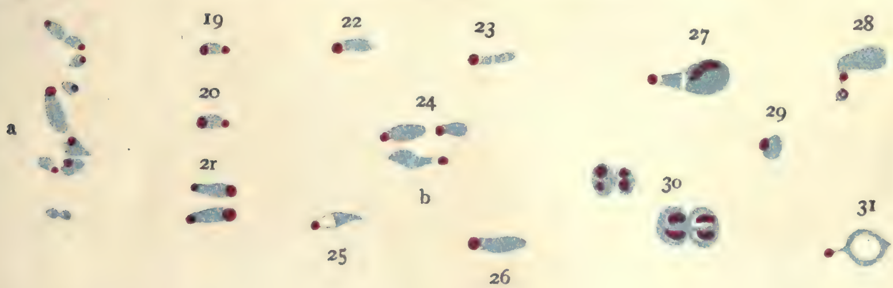


PLATE X

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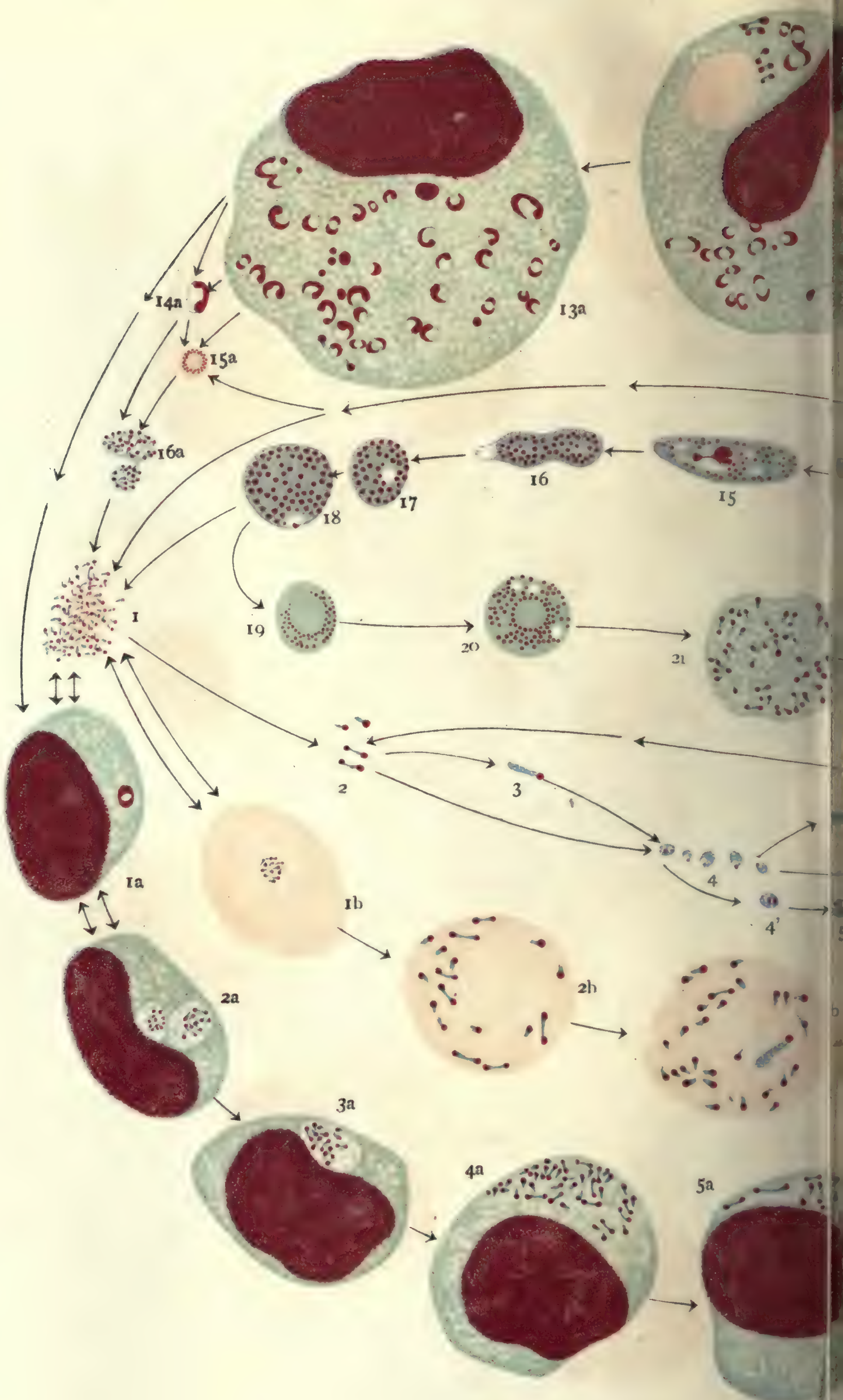
Plate III



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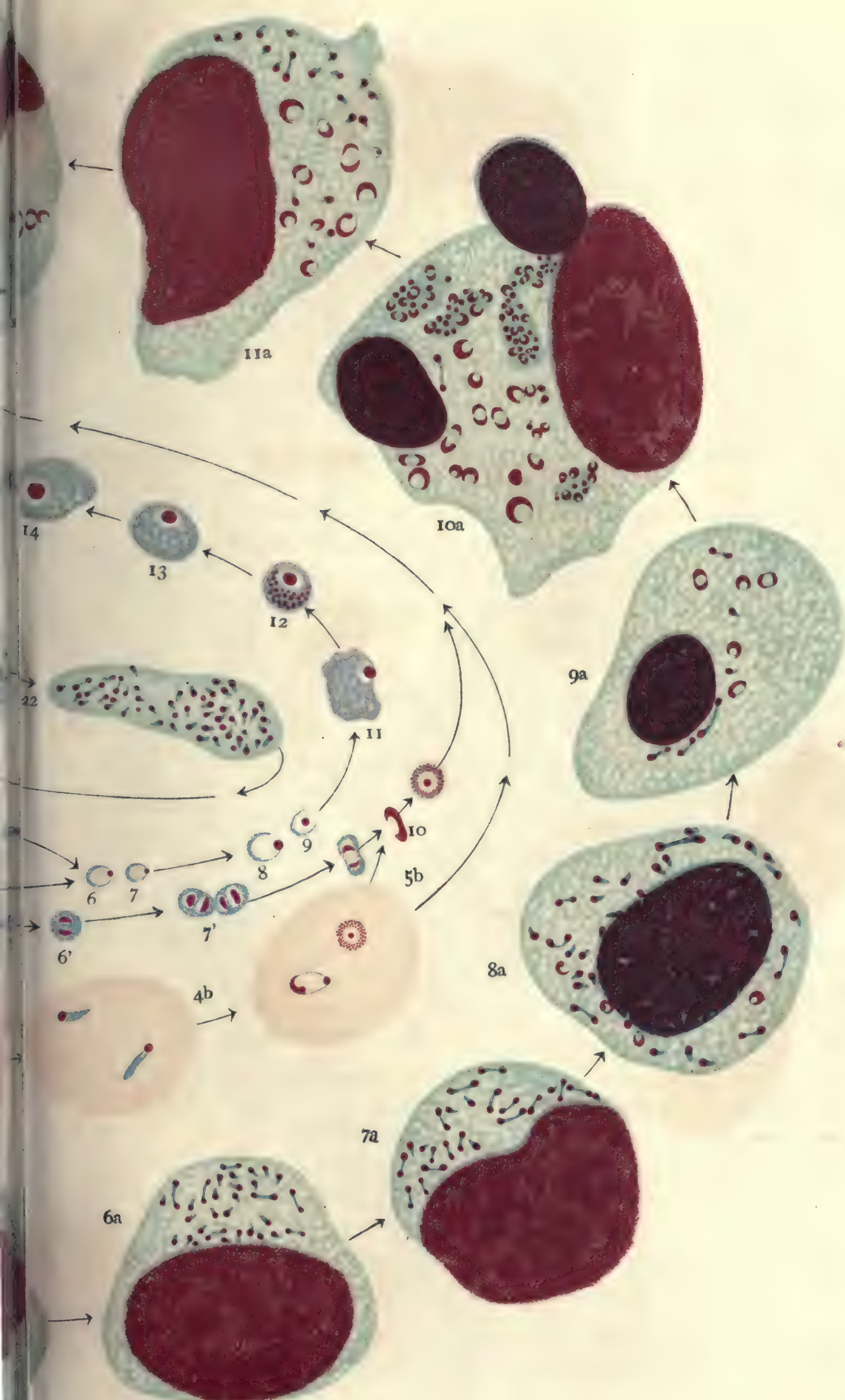

XI

THE EGG LAYING HABITS OF CALIFORNIAN ANOPHELINES *

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During the months of May, June and July, 1920, the writers were stationed at Vina, Tehama County, California, in the central part of the Sacramento Valley, where a temporary summer laboratory was established for the purpose of investigating a number of problems concerning malaria and anophelines, particularly their egg laying habits, with which this paper deals. The information herein published will in a measure supplement, confirm and correct previous observations in this field.

The species dealt with were *Anopheles occidentalis* D. & K., *Anopheles punctipennis* Say and *Anopheles pseudopunctipennis* Theobald. The synonomy of the first mentioned mosquito, *A. occidentalis* D. & K., is somewhat obscure in that it was separated from *A. quadrimaculatus,* which it closely resembles, by Dyar and Knab in 1906, and its range was listed by them as "western United States, from Southern California to Alaska, eastward thru Canada to Maine." However, Howard, Dyar and Knab (1917) in their monograph point out as an additional note that Say's type specimen for *A. quadrimaculatus* has as its locality, "Northwest Territory," which discovery makes the four-spotted anopheline of the Pacific coast, *Anopheles quadrimaculatus,* submerging their name, *Anopheles occidentalis,* and making the Eastern species *A. guttulatus* Harris. This revised synonomy has not come into general use, however, and with the above explanation offered to avoid future confusion we shall use the name *quadrimaculatus* to refer to the Pacific coast species.

The mosquitoes used in the observations herein described were captured in shell vials at a number of stations in the vicinity, such as bridges, stables, outhouses and dwellings that were visited regularly each day. The captured mosquitoes were transferred to wide mouthed pint jars partly filled with water and covered with bobbinet. The jars were placed in rows on a glass shelf supported at its corners about six inches above the surface of a laboratory table. Thus elevated and resting upon the glass shelf an incandescent electric lamp could be placed directly beneath the jars for purposes of illumination and greatly facilitated observations from above, particularly in counting the eggs.

* Contribution from the laboratories of Entomology and Parasitology, College of Agriculture, University of California, Berkeley, California.

The water used in the jars at all times was taken from a deep well and showed a uniform alkalinity of 1840 parts per million expressed in parts of $CaCO_3$. The fauna and flora of the water was not determined but was practically negligible owing to the great depth of the bored well. Contamination that ensued later was undoubtedly uniform as all jars were concentrated in a small area not over 12 by 28 inches.

The temperature of the room in which these observations were made was taken on a recording thermometer. The record of this instrument showed at the expiration of the work that the maximum and minimum temperatures were 88° F. and 66° F., respectively, and that the average of each was 76° F. and 68° F. Here again there could have been no variation in the temperatures of the several jars.

Time of Deposition.—A total of sixty-five layings was recorded from May 17 to July 11. On thirty occasions we were able to obtain the exact or approximate time of oviposition. Of these, thirteen layings were made between nine and eleven in the evening, nine between eleven and daybreak, seven between sunset and nine p. m. and four during the afternoon. It must be understood, however, that artificial conditions may have seriously influenced these findings when it is considered that the light was never as intense in the laboratory as it would have been out-of-doors, that wind exercised little influence, and that the temperature curve showed a lag of approximately two hours as compared with out-of-doors as well as a distinct moderation.

The factor or factors governing the time of egg deposition are not known, but it would seem that light, temperature, humidity, and wind are probably important considerations. It is interesting in this connection that *Anopheles punctipennis* repeatedly deposited eggs in the full glow of a 40 watt tungsten lamp at a distance of about seven inches from the egg laying insect. It is safe to assume, however, that in these instances the insects oviposited in spite of the light conditions rather than because of them. That diffuse light or darkness is the normal condition during which eggs are deposited, is well illustrated by the fact that on June 3 the lights were left on from dusk until ten p. m., during which time there had been no oviposition. At that time the lights were turned off for ten minutes and at 10: 10 when the lights were switched on it was noted that three Anophelines had deposited eggs, none of them resuming until the light was again extinguished. The daylight records of oviposition appearing in our notes are without exception for overcast, humid days when the light intensity in the house approximated that normally occurring at dusk. The range of temperature between different oviposition periods was so great as to suggest but slight effect at best unless the stimulus should arise from

a change of temperature as from warmer to cooler and vice versa. Unfortunately, no humidity records were kept. Further work with this factor in consideration seems to offer a promising field.

Method of Oviposition.—Owing to the reluctance of *A. quadrimaculatus* to oviposit in the presence of light and the scarcity of *A. pseudopunctipennis* our observations regarding the actual process of deposition are limited to but one species, *A. punctipennis.* The only reference in the literature concerning this process that we have been able to locate is that of Kerschbaumer cited by Nuttall and Shipley (1902). Nuttall writes: "With the exception of Kerschbaumer (1901) nobody has claimed to have observed the process of oviposition. He witnessed the process but once in Anopheles, . . . he does not, however, describe the process (excepting in so far as he says the insect rested directly upon the water)."

On the evening of June 4 a specimen of *Anopheles punctipennis* was seen to behave in a rather excited manner, resting for a few moments on the surface of the water and then flying to the bobbinet or sides of the jar, remaining in each position only a few seconds. She finally came to rest for several minutes on the surface and assumed a position with the abdomen more or less parallel with the surface of the water, the wings held in the normal position with relation to each other but elevated at least the width of the body above the abdomen, the posterior end of which, comprising the last two segments, was tilted upward slightly. All six tarsi rested on the surface of the water, the middle pair being lifted above the body from time to time.

At 9:46 p. m. the first egg was deposited. This was accomplished by a rather nervous jerk of the abdomen following which an egg was seen to be protruding in a vertical position from the abdomen with its convex side directed to the rear. This position was held for four seconds when another convulsive downward twitch freed the egg from the abdomen, and as the latter was returned to its former position, another egg protruded and slipped instantaneously into the vertical position as the tip of the abdomen regained its original attitude. This procedure was continued for 19 minutes until a total of 174 eggs had been deposited. The deposition of the individual eggs took place at remarkably regular intervals of from six to seven seconds. During the entire operation the female remained motionless except for the monotonus jerking of the abdomen. At the conclusion of oviposition the mosquito remained without changing position for eight minutes, after which she slowly moved off to the side of the jar, scattering the eggs with her legs as she went. Numerous statements, based evidently on the remarkable patterns assumed by the eggs on the surface, appear in the literature regarding the method of placing the ova. Grassi,

quoted from Nuttall and Shipley (1901), stated that the eggs of *A. maculipennis* were deposited in pontoons, while those of *A. bifurcatus* were laid in star shaped patterns. In the above example, however, and in many subsequent observations of the same species, the eggs were seen merely to pile up in a heap beneath the insect, toppling over as the mass became top heavy and arranging themselves in various patterns dependent upon mutual adhesion and surface tension. At the time of deposition the eggs are pearly white, becoming progressively yellowish, then darker, until at the end of about thirty-five minutes the color becomes distinctly leaden, and in about forty-five minutes they appear dull black and under the microscope are a rich chitinous brown.

Number of Eggs Deposited.—Grassi, quoted from Nuttall and Shipley (1901), states that *A. maculipennis,* the European representative of our *quadrimaculatus-guttulatus* group, deposits 100 eggs, while Hindle (1914) dealing with the same species places the number at from 40 to 100. Howard (1900) referring merely to Anopheles (no species given) also gives the range from 40 to 100. Our observations point to a considerably larger number per laying. It is impossible at the present stage of our investigations to estimate the total number of eggs laid during the life of an Anopheline as we have not been able to start our series with bred females. Our experimental insects were invariably captured specimens concerning whose previous oviposition history we, of course, have no record.

Twenty-nine specimens of *A. quadrimaculatus* deposited thirty layings totalling 6,282 eggs, in lots ranging from 140 to 315 eggs each, bringing the average per laying to 209 eggs. Thirty-three females of *A. punctipennis* in thirty-three layings, ranging from 83 to 321 eggs each, deposited 6,700 eggs; making the average per laying 203 for this species.

Our records of oviposition in *Anopheles pseudopunctipennis* are extremely limited. We were able to obtain only four females during the course of the work. Of these, two oviposited, one a total of 157 eggs and the other but 55, bringing the average to 106 eggs per female.

Considering the females of all species under observation, 38.4% oviposited in captivity. *Anopheles occidentalis* females showed an oviposition percentage of 48.3%, *A. punctipennis* 31.2% and *A. pseudopunctipennis,* based on only four specimens, 50%. These figures are not, of course, indicative of what the particular species may do in natural surroundings as our specimens, as already stated, were captured females, unfed in captivity in the majority of cases and whose opportunities for feeding before capture were unknown.

It is pertinent at this point to make a statement regarding the number of batches of eggs deposited. In the course of the work we had several cases where the females oviposited on two consecutive nights. In such cases the two layings were recorded as one. One specimen, *A. quadrimaculatus,* no 61, deposited two true batches of eggs. In this case the female was captured under a bridge on June 9 and during the afternoon of June 12 she deposited 218 eggs. On June 13 she was given a meal of human blood and on June 19 deposited 140 eggs, dying on June 20. Both batches of eggs were hatched on the morning of June 15 and 21, respectively. On numerous occasions, dissections of females that had oviposited and been fed showed the ovaries completely filled with well developed eggs. Numerous observers have stated that Anopheles may deposit several batches of eggs with a single fertilization and a blood meal for each complement of eggs. The exact number of batches and the length of time over which they are deposited needs further observation. Accurate information on this particular point is highly desirable and might change present emphasis in control work.

The hazards of life in captivity probably affected oviposition, many dying thru accident by getting "spraddled" in the water before they were ready to oviposit. Fully fifty per cent. of those dying without ovipositing showed the presence of complements of well developed eggs upon dissection. The average length of life for unfed *A. quadrimaculatus* in captivity, disregarding their probable length of life before capture, was 4.5 days and for the fed specimens 8.5 days. For *A. punctipennis,* the length of life for unfed specimens under the same conditions was 4 days and with food, 6.3 days.

Morphology of the Eggs.—In comparison with the extensive work that has been done on the morphology and classification of the other stages of the anopheline life cycle, little has been done with the eggs. The ease of classification (at least for the three Californian species) by means of egg characters recommends this line of study to workers in other localities. The characters found to vary in such a manner as to make identification simple, are length of the egg, and position and length of the float. The consideration of one or more of these factors is sufficient to place the egg of local species correctly, but in a larger group it would be necessary to utilize other characters, such as the "frill" of Stephens and Christophers (1908), a feature omitted in most of the illustrations of anopheline eggs, which encircles the flat or upper side of the egg, the formation of the floats, or the reticular membrane enclosing the egg.

These authors classify anopheline eggs into three groups: 1. those with the lateral floats not touching the margin; 2. those whose lateral

floats overlap the upper side of the egg; 3. those without floats. According to this classification *A. quadrimaculatus* and *A. punctipennis* fall in the second class and *A. pseudopunctipennis* in the third.

The egg of *A. quadrimaculatus* is fusiform, slightly rounded at each end, and tapering to the extent that one end is slightly broader than the other. The upper surface is flattened with a slight longitudinal concavity while the lower surface is broadly convex, the convexity becoming more pronounced at the broad end of the egg. The upper surface is granular, bordered by a laterally striated frill 16μ in width, except at the floats, while the lower surface shows, under proper light, a silvery reticulation. Medianly placed are two roughly oval lateral floats, each divided in a majority of cases into twelve scalloped compartments. The larger part of the area covered by these floats is on the lateral faces of the egg, but they project dorsally over the margins which are described as "gunwales" rather aptly by one author who

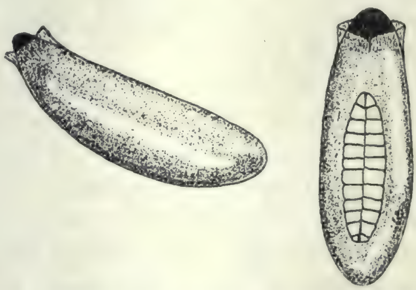

Fig. 1.—Illustrating the egg of *Anopheles pseudopunctipennis* Theobald. At the left, lateral view, and at the right, dorsal view.

likens the egg to a boat. The eggs range in length from 592 to 656μ. The floats vary in length from 144 to 224μ.

The eggs of *A. punctipennis* resemble those described above with these exceptions, the upper flattened surface is distinctly concave longitudinally, the floats are decidedly wider, projecting dorsally over the margins to the extent that they more nearly meet on the dorsal median line than those of *A. quadrimaculatus*. The floats also include approximately eighteen scalloped compartments each and extend along the sides for slightly more than one-half the entire length of the egg, while in *A. quadrimaculatus* the floats extend for only one-third the length. The range in length of the eggs of *A. punctipennis* extends from 544 to 576μ while the float length remains fairly constant at 320μ.

In *Anopheles pseudopunctipennis* is found a peculiar specialization represented in the general characters of the egg of *A. turkhudi* Liston, which is placed by Stephens and Christophers (1908) in class three of their table as lacking floats, altho vestiges are present. The eggs

are shorter than either of the two already mentioned, ranging from 512 to 528μ. The upper surface is nearly flat, showing little concavity longitudinally altho the lower surface shows a marked convexity. Both ends of the egg are rounded, one being considerably broader than the other. The floats are represented by a fusiform closely appressed area, approximately 270μ long, lying on the dorsal side of the egg and nearer the blunt end. This area is divided medianly by a line which is assumed by the authors to be the line of contact of the two floats that have been forced up from the sides. Lateral lines mark off each longitudinal half of the area into twelve sections representing the twelve original compartments of lateral floats. This area is so appressed that its position is not distinguishable from a lateral view.

Near the narrow end of the egg the membranous covering flares out from the body of the egg to form a translucent, striated collar which completely encircles the end, with the exception of a triangular incision down the dorsal median line in a manner which reminds one of an "oversized dress collar" (Fig. 1). The egg hangs at an angle in the water, supported by surface tension on this "collar." The larvae, however, unlike those of *Anopheles turkhudi,* whose eggs those of *pseudopunctipennis* resemble, retain the horizontal position at the surface of the water.

Selection of Breeding Places.—Much has been written regarding the type of breeding places frequented by various species of Anophelines and experienced observers in this field are able to forecast with a high degree of accuracy the species that they will find breeding in a given situation. This intuition is almost impossible to analyze and attempts to work out the ecology have yielded as yet only partial explanations. Disregarding the causes that make particular pools acceptable or unfavorable for the life of the larvae, there remains a fundamental question on which the whole study depends. This is, the determination of whether the selection of the particular pools is due to selective oviposition on the part of the female or the inhibiting effects, chemical or biological, upon the larvae present in some pools which are unfavorable to the species under consideration.

In the course of our work we found pools from which there constantly emerged, in the one case *A. quadrimaculatus* and in the other *A. punctipennis* with no mixture of the species. These pools were therefore classified as *quadrimaculatus* pools or *punctipennis* pools. Eggs of each species were "planted" in the pools hitherto inhabited only by the larvae of the other species and their development observed. In order to accomplish this under the most natural conditions, "lug" boxes with bobbinet coverings substituted for bottoms were inverted in the pools, supported a little from the bottom by stakes

and reaching an equal distance above the surface to prevent overflow. By supporting them a little from the bottom it was hoped that the natural enemies might successfully enter and that the enclosed water would partake of all the conditions prevalent in the pool and still not allow the escape in any appreciable numbers of the surface feeding larvae. Through an opening in the bobbinet covering, the eggs were gently washed on the surface of the water in the box. Unfortunately our boxes were of necessity located in pools subject to the rise of a creek, an occurrence that happened several times in the night owing to thunder storms in the mountains and the unexpected flow of unused irrigation water. Due to this contingency all of our boxes with the

Fig. 2.—Showing manner in which lug boxes were placed in a typical *A. punctipennis* pool to determine suitability of this pool to other species of anophelines.

exception of one set were found on one or more mornings to be awash, rendering their results problematical. One set, however, was conducted under optimum conditions. A pool, six by twenty-five feet in area known in our experiments as a *punctipennis pool,* was formed by the receding creek mentioned above and fed by seepage and a trickling connection with the main stream. It was shaded, cool and thickly overhung with surrounding brush, mainly grape, cottonwood and sycamore. The bottom was made up of water-rounded stones ranging in size from pebbles to small boulders and its prominent vegetable growth was a member of the Crenothrix group. The water was unusually clear and had an alkalinity of 840 parts of $CaCO_3$ per

million. It showed evidence of being permanent, in part at least, throughout the summer. Two boxes were installed as mentioned above (Fig. 2) enclosing a section in which larvae had been observed and removed. On June 28, 474 eggs of *A. quadrimaculatus* were placed in one box and on July 1, 635 eggs of *A. punctipennis* were placed in the other. These eggs were observed to hatch in the normal period in both boxes and daily observations proved their gradual development, pupation beginning on the thirteenth day after egg deposition. No accurate count of the numbers emerging was possible under the existing conditions, but from general observation, no retardation in development or diminution in the expected numbers of *A. quadrimaculatus,* altho breeding in an *A. punctipennis* pool, could be noted. The results of this experiment left to our minds only two alternatives in the question of selective breeding places—either the *punctipennis* larvae are cannibalistic on the *quadrimaculatus* larvae when the former are in optimum surroundings, and the process is reversed when optimum conditions are furnished to *quadrimaculatus* larvae, or what is far more likely—the female exercises selection in depositing her eggs. Several experiments were inaugurated to settle this first alternative by mixing the eggs in one box but the floods mentioned above rendered our results untrustworthy.

Incubation Period.—In the regular routine of laboratory work, the jars were examined every morning at about nine o'clock, the eggs that were found for the first time being entered as deposited for that day and those found to be hatched were entered at the same time. By observing this routine the average incubation period was very nearly approximated. For *A. quadrimaculatus* the average incubation period was 2.5 days with a range from 2 to 4 days. The eggs of *A. punctipennis* averaged 3.2 days with a range from 2 to 6 days. Temperature is quoted by many authors as distinctly influencing the length of the incubation period. With many of our sets, however, laid on the same day and subjected to the same conditions a considerable amount of variation was recorded. It seems highly probable that temperature should exercise a decided effect on incubation particularly at the extremes but within a certain range such as our eggs were subjected to, i. e., 68° to 76° F., little effect could be noted.

Desiccation Experiments.—Mitchell (1907) states that Dr. Dupree "has had the eggs of *Anopheles* develop after as many as ten hours out of water, this, however, being exceptional." Stephens and Christophers (1908) state anopheline eggs removed from water, placed on paper and allowed to dry for more than two, or, at the most three days, will not hatch if they are kept at a temperature of 86° to 96° F. In an attempt to check these findings for the Californian species, eggs

of *A. quadrimaculatus* and *A. punctipennis* were removed from the water six hours after they had been deposited by allowing them to adhere to the surface of a strip of filter paper dipped among them, leaving a number of eggs in the jar as a check. The filter paper was then suspended by pins inside a capsule box and allowed to dry out at room temperature. Drying was accomplished in a remarkably short time for at the end of four hours the paper was entirely dry and the eggs rattled off its surface at the least movement. At intervals of twenty-four hours a supply of dried eggs were taken from the filter paper and placed in shell vials of tap water. We were never able to obtain a hatch from eggs of *A. punctipennis* that had been removed from water for twenty-four hours. However, with *A. quadrimaculatus* eggs removed from the water on June 14, dried and replaced on the fifteenth, sixteenth and seventeenth, having been subjected to drying for 24, 48 and 72 hours, respectively, there were produced excellent hatches on the seventeenth, eighteenth and nineteenth, showing not only that the eggs of this species can withstand drying for these periods, but also the rather interesting fact that egg development ceases as soon as they are removed from the water. Eggs from this lot placed in water on the eighteenth (96 hours of drying) and for several succeeding days failed to hatch. The maximum and minimum temperatures to which the eggs were subjected during this period were respectively 74° F. and 65° F. Another attempt to duplicate this set of experiments with the same species and technique when the temperature ranged between 70° F. and 80° F. resulted in the failure of the eggs to hatch after 48 hours of drying.

The authors present this paper not as a complete treatise on egg deposition of Anophelines but as observations that may add to the general fund of information concerning these insects whose activities are of such vital interest and importance to mankind in all parts of the world. The authors wish to acknowledge the helpful co-operation and limitless enthusiasm in this work on the part of their two student assistants, Mr. Clifford T. Dodds and Mr. John F. Lamiman of the University of California.

SUMMARY

1. The process of egg deposition in *Anopheles punctipennis* is described.

2. The number of eggs deposited per laying is found to be greater than hitherto recorded, *A. quadrimaculatus* averaging 209 eggs and *A. punctipennis* 203 per laying.

3. Descriptions are given of the eggs of the Californian anophelines whereby they may be differentiated, including a description of the

egg of *A. pseudopunctipennis,* which represents a marked departure from the usual anopheline type.

4. Observations are introduced to indicate that specific breeding places are due to selective oviposition.

5. The incubation period of the eggs of *A. quadrimaculatus* is 2.5 days, and *A. punctipennis* 3.2 days, and *A. pseudopunctipennis,* 3 days.

6. It was found that the eggs of *A. quadrimaculatus* could withstand drying for 72 hours but that those of *A. punctipennis* failed to hatch after 24 hours of drying.

Literature Cited

Dyar, H. G., and Knab, F. 1906.—Notes on Some American Mosquitoes, with Descriptions of New Species. Proc. Biol. Soc. Wash., 19: 159.

Hindle, Edward. 1914.—Flies in Relation to Disease, Blood Sucking Flies. Cambridge, University Press, London.

Howard, L. O. 1900.—Notes on Mosquitoes of the United States. Bull. 25, Bureau of Entomology.

Howard, L. O., Dyar, H. G., and Knab, F. 1917.—The Mosquitoes of North and Central America and the West Indies. Vol. IV. Publication 159, Carnegie Institution of Washington, D. C.

Mitchell, E. G. 1907.—Mosquito Life. New York.

Nutall, G. H. F., and Shipley, A. E. 1901.—Studies in Relation to Malaria. Jour. Hyg., 1: 50.

1902.—Studies in Relation to Malaria. Jour. Hyg., 2: 65.

Stephens, J. W. W., and Christophers, S. R. 1908.—The Practical Study of Malaria. University Press, Liverpool.

ON THE MIGRATORY COURSE OF *TRICHOSOMOIDES CRASSICAUDA* (BELLINGHAM) IN THE BODY OF THE FINAL HOST*

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Trichosomoides crassicauda, a nematode belonging to the family Trichinellidae, was found in the bladder of wild rats in 1845 by Bellingham. Hall (1916) has summed up the previous work on this species. This nematode is remarkable on account of the great difference in size between the males and females. The male is commonly found parasitic in the vagina or uterus of the female, having a length of 1.46 to 2.5 mm. and a width of 23 to 33μ. The female is 10.5 to 13 mm. long and attains a maximum width in the posterior region of the body of about 0.2 mm. Nothing is known of the method of infection of this parasite or its migratory course to the bladder of the rat. Von Linstow (1874) suggested that the embryos might bore into the wall of the digestive tract and make their way to the pelvis of the kidney by way of the renal artery. He described also that sexually immature individuals, the males but little smaller than the females, were found in the pelvis of the kidney and that copulation took place in the ureters. Later the females became larger and the males entered their vaginae. Hall (1916: 16) found that the embryos escaped from their shells in the vagina of the female after the worm had been in normal salt solution a short time. He suggested from this observation and from the fact that such embryos seemed to live only a short time that infection must take place in a rather short period as a rule, or else the embryos would perish. He also described that the embryos which had just escaped from the egg, had a body of almost uniform thickness, terminating in bluntly rounded ends, while von Linstow stated that they were provided anteriorly with a single lancet-like process.

I found some specimens of *Trichosomoides crassicauda* in wild rats *Epimys norvegicus,* collected near Baltimore during the winter of 1919-20. The parasites were usually found attached to the wall of the bladder with their anterior ends somewhat embedded in the mucous membrane. They cause a rather high degree of catarrhal cystitis according to the number of the parasites present. The mucous membrane of the bladder was found congested and swollen and the urine

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* Contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

more or less muddy. The number of the parasites in the bladder of a single rat is commonly small, although sometimes ten or more specimens were found in a single host.

In order to discover the method by which the rat is infested with *Trichosomoides crassicauda* I fed two white rats on January 20, 1920, with the eggs which contained the fully developed embryos. One of these rats became infected and the parasites developed to maturity. The eggs from the urine of this rat were used in later experiments. After the success of this experiment I undertook to investigate the migratory course of the larvae in reaching the bladder of the rat by feeding white rats with large numbers of the eggs of this parasite, and examining the various organs a short time after feeding. Three rats were used at different times but from only one of them were the larvae recovered. The results of this study are incomplete and a much larger series of experiments will be needed to clear up the details of the problem. However, some very definite information was obtained in regard to the migratory course of this parasite in the body of the final host. Since it will be impossible for me to continue these experiments I am writing up my results in hopes that some one else may use them in carrying on further work on this interesting problem.

Since the methods used with the three experimental rats were the same, I will describe here only the details of the work with the one rat which gave positive results.

On May 15 and 16, I fed one half grown white rat with many eggs of *Trichosomoides crassicauda* collected from the urine of the white rat experimentally infected with the parasite and of two wild rats which were infested naturally. The next day I fed it 15 adult worms containing many eggs in their uteri and also many eggs collected from the urine of the three rats mentioned above. On the following day also it was fed with many eggs collected from the urine of these rats. I killed this rat on May 19, one to four days after the various feedings.

The following technic was used in the recovery of the larvae from the body of the rat. First I opened carefully the abdominal cavity, avoiding bleeding, and washed it out several times with normal saline for the purpose of collecting any larvae contained in it. The liver and other internal organs were found to be a little congested without showing any bleeding points. Then I opened the pleural cavity in the same way and washed it out with normal saline. Both lungs were found to be a little congested showing some bleeding points on their surfaces. I examined one third of the liver, both kidneys, the spleen, and the left lung according to the following method. These internal organs were crushed into fine pieces and washed with normal saline

two to four times, and filtered through a fine wire net. The filtrate from the different organs and wash water of abdominal and pleural cavities were kept carefully separate and were centrifuged to collect the larvae.

The following results were obtained from these examinations. Four larvae were found in the abdominal cavity, two in the pleural cavity, and three in the left lung. One third of the liver, both kidneys and the spleen were examined without finding any larvae, while the right lung and two thirds of the liver were preserved for later microscopical examinations. I also examined the ureters, bladder and heart, without finding any larvae. Comparing the structure of the young larvae found in the body cavity and in the lungs with larvae just escaped from the eggs, I learned the following facts. The larvae just from the eggs have a very small body of almost uniform thickness, terminating in bluntly rounded ends. They measure about 0.21 to 0.25 mm. in length and 8 to 10μ in thickness. It was impossible to make out any details of internal structure at this stage. The larvae found in the abdominal cavity of the experimental rat measured 0.82 to 0.84 mm. in length and 34 to 35μ in width. The anterior part of the body was a little thicker than the posterior end and ended bluntly. The digestive tract was not very clearly defined. The posterior end of the body was blunt with a diameter of about 0.02 mm. and had a small depression. The esophagus consisted of about twenty irregularly shaped cells. No esophageal cells were present for about 0.02 to 0.025 mm. at the anterior end. The esophagus was relatively long, measuring about 0.28 mm. The intestine ran straight along the middle body toward the posterior end and consisted of cells containing fine granules. The larvae found in the pleural cavity and two of the larvae found in the lungs were larger than those from the abdominal cavity. They were about 2.34 mm. long and 0.1 mm. thick. The anterior end was rounded and 0.03 mm. in diameter. The width increased toward the middle of the body with a maximum of 0.1 to 0.11 mm. and then decreased posteriad. The esophagus consisted of about twenty cells and measured about 0.54 mm. in length. No cells were present for a short distance at the anterior end. One larva found in the lung was smaller than the others found in the same location, having a similar size and structure to the larvae found in the abdominal cavity. It is very probable that the larger larvae are females and the smaller ones males.

From these experiments I learned the following facts (1) Infection with *Trichosomoides crassicauda* can be induced in the white rat by feeding the eggs of the parasite. (2) The adult worms found in the bladder of experimental animals are very few compared with the

number of eggs swallowed. (3) The eggs swallowed by the final host hatch in the digestive tract and penetrate through its wall into the abdominal cavity. From here they travel into the pleural cavity probably through the diaphragm and penetrate into the lungs from their surfaces.

The passage of the larvae of *Trichosomoides crassicauda* into the lungs of the experimental rat is very interesting in relation to the recent work on the life history of *Ascaris lumbricoides* which proves that after hatching in the intestine of the final host the larval stages of this parasite must make their way to the lungs before they can complete their development. Yoshidi (1918) and Ransom and Foster (1920: 30) conclude that this phenomenon is of common occurrence in the life cycles of parasitic nematodes. Besides the hookworms, Strongyloides, *Ascaris lumbricoides, Ascaris suum* and *Belascaris marginata,* they note also that *Haemonchus contortus* from the sheep and *Ascaris anoura* from the python probably have a lung phase in their life cycles. Recently Neshi (1918) reports the finding of four larvae of *Trichuris depressiuscula* in the lungs of a dog twenty-one hours after experimental infection.

This observation and my finding the larvae of *Trichosomoides crassicauda* in the lungs of the experimental rat after feeding with the eggs add the family Trichinellidae to those in which this phenomenon occurs, and strengthens Ransom and Foster's hypothesis that parasitism of the lungs by nematodes is a more primitive condition than parasitism of the alimentary canal.

On the method of migration of the larvae of this nematode from the lungs to the bladder of the host there is little definite information. Von Linstow's (1874) finding young worms in the kidneys and ureters suggests that they make their way to the kidneys and then pass down the ureters to the bladder. How they make their way from the lungs to the kidney is still an unsolved question. Taking for granted that the migration to the lungs is a necessary phase of the life history of this parasite, there are three possible ways in which it might migrate from the lungs to the kidneys. (1) The larvae might make their way into the small branches of the pulmonary veins, be carried to the heart and then pass to the kidney along the aorta and renal arteries. (2) The larvae in the lungs might break into the air cells and, like the hookworm larvae, travel up the trachea and down the esophagus into the intestine. From here it would be necessary for them to make their way to the kidneys by way of the body cavity. (3) Finally the larvae in the lungs might make their way back into the pleural cavity, through the diaphragm and body cavity to the kidney. The course by the blood stream would seem to be very difficult if not

impossible on account of the large size of the larvae and the fact that the renal artery is a small vessel which branches at right angles from the dorsal aorta. It is possible of course that the smaller type found might follow this course. It seems to me that the second course is the most probable, but the solution of this interesting problem must await future investigations. At any rate the passage to the kidney requires a prolonged and difficult migration. This may account for the difficulty of producing the infestation and why the number of worms found in the bladders of the wild rats is usually so few.

SUMMARY

1. The infestation of rats with the bladder nematode, *Trichosomoides crassicauda,* was accomplished by feeding the eggs.

2. The finding of larvae of this species in the body cavity, pleural cavity and lungs of an experimental rat fed with large numbers of eggs, suggests that in the life cycle of this species the larvae must pass to the lungs before they can establish themselves in their normal habitat.

3. This observation and other recent studies strengthen the view that migration to the lungs is a common phenomenon in the life cycle of nematodes.

4. How larvae reach the bladder from the lungs was not determined but they probably are not carried in the blood vessels.

I wish to express here my thanks to Dr. W. W. Cort, under whose direction this work was carried on, for his help in the course of this investigation and for revising the manuscript.

Literature Cited

Hall, M. C. 1916.—Nematode Parasites of Mammals of the Orders Rodentia, Lagomorpha, and Hydracoidea. Proc. U. S. Nat. Mus., 50: 1-258.

Neshi, G. 1918.—Ueber die Entwicklung des Trichocephalus innerhalb des Wirtes. (Japanese.) Tokyoer Med. Wochr., No. 2080.

Ransom, B. H., and Foster, W. D. 1920.—Observations on the Life History of *Ascaris lumbricoides.* U. S. Dept. Agr. Bull. 817.

Von Linstow, O. 1874.—Beobachtungen an *Trichodes crassicauda* Bell. Arch. Naturg., 1: 271-286; 1 pl. (Cited after Hall, 1916.)

Yoshida, S. 1918.—The Migratory Course of the Larvae of Ascaris. (Japanese.) Byo ri Gaku Zasshi, Vol. 8.

NOTES ON *NOSEMA APIS* ZANDER*

R. Kudo

Aside from *Nosema bombycis* Nägeli, no other Microsporidian has received so much attention of investigators as *Nosema apis* Zander. A disease of adult honey bees for which the Microsporidian is responsible, and which is known by different names such as Nosema-Seuche, Isle of Wight disease, Nosema disease, etc., has been reported to occur in various parts of the world. In North America, White (1914) found 40 infected bees in 120 samples received from 27 states of the union, and two diseased samples received from Canada. The same author further published (White, 1919) valuable results of experiments on the means by which the spores may be killed.

The morphology and development of the Microsporidian have, however, been studied by but three investigators, i. e., Zander (1911) and Fantham and Porter (1912). According to these authors, the spores of *Nosema apis* are on the whole similarly constructed to those of *Nosema bombycis* studied by Stempell (1909). Recently, I had an opportunity of studying the Microsporidian, and have obtained more or less different results by observations upon the structure of the spore from those of the above mentioned European investigators. I shall briefly describe the results in the following pages.

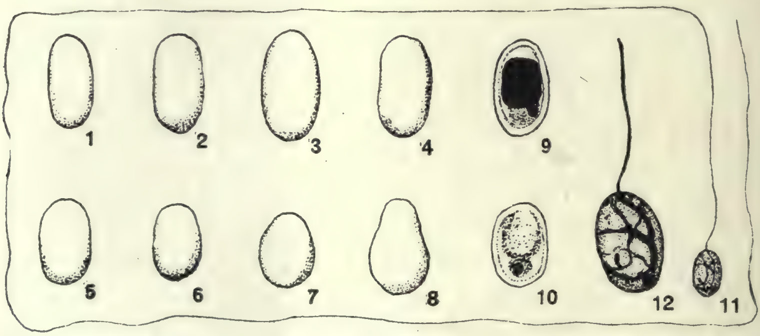
In a field at Spring Valley, New York, 660 workers of *Apis millifica* were collected from August 27 to September 5, 1920. Although I could not trace the hive for these captured bees, there were a number of hives in the nearby woods where the collection was made. The digestive tract of each bee was drawn out from the body by means of a pair of forceps and a needle. A part of the ventriculus was smeared on a slide and examined microscopically in the fresh state.

Out of 660 bees thus examined, 25 were found to be infected by *Nosema apis*. After encountering one or two heavily infected bees, it was not difficult to diagnose a heavily infected host insect by its inactivity and peculiarly softened abdomen. When a part of a heavily infected ventriculus was placed on a slide, the milky white appearance gave a definite sign of the nature of the infection before a microscopical examination was made. Four bees contained spores similar to those of *Nosema apis*, differing, however, in larger dimensions and a large clear rounded space at one extremity. The presence of a

* Contribution from the Zoological Laboratory of the University of Illinois, No. 167.

space which is generally called a vacuole, in the mature spores, recognizable in fresh state, has commonly been noted in many species of Microsporidia. Frequently the spores of *Nosema apis* show also a clear rounded area near at one end, which, however, does not occur so regularly as in the spores from the above mentioned four bees. Whether this form should be distinguished from *Nosema apis* by a different specific name or not will have to be determined by a comparative study on the various stages of their development.

The dimensions of spores of *Nosema apis* differ somewhat widely according to different authors. According to Zander (1911), the spore meausres 5μ in length and 2.86μ in Dreadth, and the polar filament seemed to be shorter than that of *Nosema bombycis* measured by Stempell (1909). Fantham and Porter (1912) recorded the length and breadth 4 to 6μ (up to 7μ) and 2 to 4μ, respectively, and stated



Spores of *Nosema apis*. Figs. 1-8. Fresh spores of various form and size. × 2350. Fig. 9. A spore stained with Heidenhain's iron hematoxylin. × 2350. Fig. 10. A spore stained with Giemsa's solution. ×2350. Fig. 11. A spore with an extruded polar filament, stained after Fontana (the polar filament is diagrammatically shown without changing its approximate length). × 1200. Fig. 12. A more highly magnified view of the spore shown in fig. 11. ×2350.

that the polar filament was "about 60μ" long. White's measurements (1919) are as follows: fresh spores in India ink smears, 4.46μ long by 2.44μ broad; stained spores, 4.15μ long by 2.06μ broad.

According to my observations, fresh mature spores vary from 4.6 to 6.4μ in length, and from 2.5 to 3.4μ in breadth and thickness. The dimensions were obtained by measuring 250 spores. As mature spores (Figs. 1-8) differ to a more or less great degree in form and size, care has been taken in choosing the spores. I have measured ten spores from a smear of each infected individual, one spore taken at random near the center in every other field.

In the fresh condition, a spore does not show any differentiation in its contents. It is slightly less refractive than that of *Nosema bombycis*. The polar filament is invisible in fresh state, as in the

majority of microsporidian spores, except such a form as *Thelohania magna* (Kudo, 1920). The presence of a polar filament in the spore, therefore, could only be proved by causing its extrusion. Zander (1911) made no effort, and mentioned simply that it seemed to be shorter than that of *Nosema bombycis* as studied by Stempell. Fantham and Porter (1912) stated that it measured about 60μ in some spores they had examined.

The polar filament of *Nosema apis* can easily be extruded and observed, although some authors are inclined to think it is "very difficult of observation under any circumstances" (Fantham and Porter, 1912: 174). Either mechanical pressure (Kudo, 1913) or perhydrol (Kudo, 1918) causes extrusion of the filament in fresh spores. For quick observation a dark field microscope is indispensable (Kudo, 1918). But for permanent preparation, Fontana's staining (Kudo 1920) gives best results according to my more recent observations.

As to the method of application of mechanical pressure upon the spores, I stated briefly (Kudo, 1913) that they were pressed by fingers between a cover glass and a slide. Since I have often been asked as to the exact technique, I shall briefly describe the method here. A very small drop of water emulsion of fresh microsporidian spores is placed on a slide by means of a fine capillary pipette and is covered with a cover glass. It is desirable to have the outer margin of the cover glass unoccupied by the emulsion. Place the slide on a smooth and steady surface, and cover the cover glass with a piece of cloth or filter paper, over which the elbow is gently applied. Give a strong downward push to the arm. This will instantly cause the extrusion of polar filaments. The slide now can be brought on the stage of a dark field microscope and examined. To make the preparation permanent the cover glass must be lifted up carefully. After being fixed with fixative such as sublimate alcohol, the smear is stained after Fontana.

The completely extruded polar filament of spores of *Nosema apis* measures from 230 to 280μ in length. The remarkable difference in the length of polar filaments compared with that obtained by Fantham and Porter, is due solely, I believe, to the difference in the technique applied. In the case of *Nosema bombycis,* I have already shown (Kudo, 1913, 1916) how different methods bring out entirely different results regarding this delicate structure characteristic of Microsporidia. Iodine water has been used by several investigators, including the above mentioned English authors, in causing the filament extrusion of microsporidian spores. Yet evidences obtained by comparative studies of various methods have shown me that the iodine water in this case is not likely to cause the extrusion, but simply stains the already extruded

polar filaments, most probably due to the pressure of the cover glass, to a more or less recognizable extent, showing, therefore, only incompletely extruded polar filaments in very small numbers.

When examined under a dark field microscope (Fig. 13), the extruded filament of *Nosema apis* shows a uniform thickness throughout, as those of other Microsporidia which I have studied up to the present. Its form is, however, very striking in some cases. Leaving the spore at one end, it shows about from 10 to 15 undulating courses of a large wave length. This part is followed by another about 10 to 15 turns of uniformly small wave length. Somewhat similar conditions were frequently noticed in the polar filament of *Nosema bombycis,* extruded under the influence of perhydrol (Kudo, 1918, Fig. 5), but it was not so conspicuous as in the present Microsporidian. As far as I am aware, this peculiar condition has never been reported to occur. This wavy form becomes straightened out gradually and loses its former appearance, although the distal portion often remains in

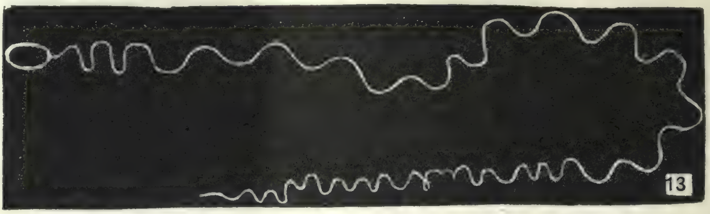


Fig. 13. A spore of *Nosema apis* with the extruded polar filament viewed under a dark field microscope. ×1200.

more wavy condition than the basal. Some polar filaments are, however, as straight as a stretched thread. The difference in the form of the extruded filament is due, in my opinion, to the difference in pressure received. It seems probable that when the spore receives a sudden violent pressure, the polar filament escapes from the spore without unwinding its coiled form, and that when the spore receives a gradually increasing pressure, the extrusion of the filament takes place slowly in a more or less straightened form. When the cover glass is removed from the slide, the agitation of the emulsion in which the spore is floating, apparently tends to straighten the filament, so that after Fontana's staining straight forms tangled in various ways are mostly to be found. Fontana's method stains not only the extruded polar filament, but also its unextruded portion. In this regard, the method has superiority over Löffler's (Kudo, 1913). Thus in the spore shown in Figs. 11 and 12, the unextruded part of the filament is clearly distinguishable, suggesting the extrusion in this particular spore was incomplete.

When the spores are fixed and stained, the contents appear more or less differentiated. In spores stained with Giemsa's solution, a small rounded sporoplasm taking a typical blue color with its deeply red nucleus, is seen near one end, while between this and the other end of the spore, the polar capsule with its polar filament is observable (Fig. 10). In spores stained with Heidenhain's iron hematoxylin, similar differentiation appears, although the polar capsule with its filament frequently stains as deeply as the nucleus of the sporoplasm (Fig. 9). The polar filament does not start to coil close to the end of the spore, to which it is attached. The basal portion is rather straight and begins to coil some distance back (Figs. 9 and 14). Zander (1911) observed this portion, but misinterpreted the relative position of the polar filament and the sporoplasm. Thus the structure of

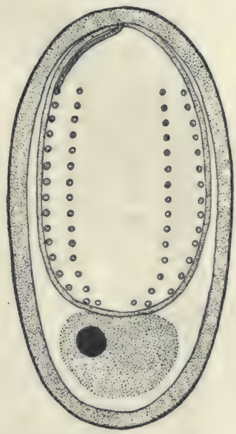


Fig. 14. A schematic representation of the longitudinal section through a mature spore of *Nosema apis*. Except for the basal portion the doubly coiled polar filament appears only in cross sections. ×10,000.

the spore of *Nosema apis* is similar to that of *Thelohania magna* (Kudo, 1920).

The fact that the polar filament of the spore of *Nosema apis* when extruded shows under certain circumstances two regions, one with a regularly large wave length, and the other with a uniformly small wave length, each having 10 to 15 turns, leads me to assume that the mode of coiling of the filament inside of the spore is a particular one. The only interpretation that can be advanced in this connection is that the polar filament of the spore of *Nosema apis* is coiled from 10 to 15 times along the polar capsule, inside of which and continuous to it, it is coiled back again toward the tip where the filament is attached. To illustrate the structure, a schematic longitudinal section of a spore is shown in figure 14.

SUMMARY

1. Among honey bees collected at Spring Valley, New York, from August 27 to September 5, 1920, 3.8 per cent. were found to be infected by *Nosema apis*.

2. Four bees harbored an undetermined Microsporidian.

3. Fresh spores of *Nosema apis* measure from 4.6 to 6.4μ long, and from 2.5 to 3.4μ broad and thick.

4. A method of applying mechanical pressure to cause the filament extrusion of microsporidian spores, is described.

5. The polar filament of the spore of *Nosema apis* is 230 to 280μ in length. The structure of the spore is similar to that of *Thelohania magna*.

6. Extruded polar filaments show two parts, one with larger and the other with smaller undulations, each composed of 10 to 15 turns, indicating that the filament is doubly coiled.

PAPERS CITED

Fantham, H. B., and Porter, Annie. 1912.—The morphology and life history of *Nosema apis* and the significance of its various stages in the so-called "Isle of Wight" Disease in bees (Microsporidiosis). Ann. Trop. Med. Parasit., 6: 163-195, 3 pl. and 1 textfig.

Kudo, R. 1913.—Eine neue Methode die Sporen von *Nosema bombycis* Nägeli mit ihren ausgeschnellten Polfäden dauerhaft zu präparieren und deren Länge genauer zu bestimmen. Zool. Anz., 41: 368-371, 4 fig.

1916.—Contributions to the study of parasitic protozoa. I. On the structure and life history of *Nosema bombycis* Nägeli. Bull. Imp. Seric. Exp. Stat. Tokio, 1: 31-51, 2 pl.

1918.—Experiments on the extrusion of polar filaments of Cnidosporidian spores. Jour. Parasit., 4: 141-147, 1 pl.

1920.—On the structure of some Microsporidian spores. Jour. Parasit., 6: 178-182, 11 fig.

White, G. F. 1914.—Destruction of germs of infectious bee diseases by heating. Bull. U. S. Dept. Agr., no. 92, 8 pp.

1919.—Nosema Diseases. Bull. U. S. Dept. Agr., no. 780, 59 pp., 4 pl. and 7 figs.

Zander, E. 1911.—Handbuch der Bienenkunde. II. Krankheiten und Schädlinge der erwachsenen Bienen. Stuttgart. 42 pp., 8 pl. and 13 figs.

ACANTHOCEPHALA PARASITIC IN THE DOG *

H. J. Van Cleave

There are few published records of the occurrence of Acanthocephala in dogs. The available accounts show considerable uncertainty regarding the identification of the species encountered. It seems probable that the infestation of dogs in southern Europe by *Moniliformis moniliformis* is accidental and as Porta (1914: 484) has shown the case reported from Calcutta is probably that of a misidentified nematode. But one species has been recorded from dogs in North America. This species seems to have greater significance as a dog parasite than those reported elsewhere in that it seems to be a normal parasite of the dog since the worms reach full sexual development in this host.

In 1909 B. F. Kaupp described *Echinorhynchus canis* from specimens collected by J. W. Parker from a dog at San Antonio, Texas. Hall and Wigdor (1918) have recently called attention to the lack of subsequent references to Acanthocephala from dogs of this continent and have placed upon record one additional instance of an infestation by a single specimen of this species. Attempting to follow the recently proposed classification of Travassos the last mentioned authors have ascribed *E. canis* to the genus Oncicola though the explanations accompanying all of their text figures cite it as *Gigantorhynchus canis*. In their text mention is made of the difficulties encountered in attempting to determine the genus to which their single immature female specimen belongs.

J. W. Parker, the collector of the specimens upon which the original description of *Oncicola canis* (Kaupp) was based, published a note containing fairly significant additions to the biology of this species in the same volume of the journal which contained Kaupp's description. According to his account about three hundred specimens of this species were obtained from the single host individual which died of symptoms strongly indicative of rabies (Parker 1909: 703). Upon post mortem "numerous ulcerations, as from abrasions three or four days old, were found on the buccal and gingival membranes and tongue;" and "about three hundred small worms (*Echinorhynchus canis*) were found in the jejunum and ileum, chiefly in the ileum, most of them attached, in some cases the head penetrating mucous and muscular coats to the peritoneum." In speaking of the possible normal host of this parasite Parker continues, "'Mad' coyotes are frequently

* Contributions from the Zoological Laboratory of the University of Illinois, No. 168.

reported in the vicinity, much more frequently than rabies is reported among domestic animals. I, therefore, think it probable that *Echinorhynchus canis* is normally a parasite of the coyote."

The writer has encountered an instance of the occurrence of *Oncicola canis* from an unusual locality. A single specimen of this species contained in the collection of Professor Henry B. Ward was taken from a dog at Lincoln, Neb., by Dr. A. D. Brewer in 1897. Reference is made to this individual by Ward (1897: 174) as *Echinorhynchus sp?,* no attempt having been made at that time to determine the species. The present writer has examined the specimen, which has full bodily development though it is not gravid. The presence of the single female worm in the intestine of the host precluded the possibility of fertilization and embryo formation. There is no indication that Oncicola has become permanently established in the vicinity of Lincoln, for of the twenty dogs thoroughly examined in securing data for the tables published by Ward only the single instance was encountered, though "among the animals which were examined were representatives of various conditions of life under which these forms are found, both the half wild strays of the city streets and alleys and the pet animals of the home."

It is impossible to determine with certainty the source of this unusual infestation. However, since the armadillo carries the larvae of *O. canis* it seems probable that either the dog must at some time have been in the South where the larvae occur or it might have been allowed to feed upon offal from armadillos brought from the South as specimens. There is little danger that Oncicola may become established as a dog parasite beyond the geographical range of the armadillo unless it has the powers of adaptation to entirely new larval hosts. However, it seems probable that this parasite is much more prevalent as a dog parasite in the southwest than published accounts of its occurrences would indicate. Consequently since the present writer has discovered additional facts regarding this species, especially with reference to one of its larval hosts, it seems worth while to publish the results of this investigation.

Two of the original specimens of *Oncicola canis* are deposited in the Parasite Collection of the United States Public Health and Marine Hospital Service (cat. no. 9409), where they were received in October, 1902. Parker makes reference to his unsuccessful attempts to secure an identification of the specimens previous to the description given by Kaupp. The two specimens submitted to the Public Health Service fortunately include both a male and a mature female. Opportunity has been afforded the present writer to examine these specimens from the original lot and he has been able to establish the identity of these

with previously unidentified larvae from the nine-banded armadillo contained in the collections of the U. S. National Museum and of the Bureau of Animal Industry. Three lots of these larvae were collected by Dr. Albert Hassall in Texas during October, 1891, (cat. nos.: B. A. I. Parasite Collection, no. 2077; Smithsonian Institution, Hassall Collection, no. 6312 and no. 6327). A fourth lot of larvae of this species bear the date of November, 1891, and were collected by Hassall from a specimen at Washington, D. C. The demonstration of the identity of these larvae with adults of *O. canis* establishes another link in the chain of the life cycle of this species. The excessively heavy infestation of the peritoneum of the abdomen of the armadillo renders the extent of the infestation in the dog encountered by Parker readily understandable.

The absolute relation of the armadillo in the life cycle of *O. canis* is not immediataely determinable. Almost without exception an arthropod serves as the primary host which ingests the passive, hard shelled embryos of the Acanthocephala. Vertebrates which shelter the larvae of these parasites usually bear the relation of intermediate host to the parasite. Hence it seems probable that the armadillo serves *Oncicola canis* as intermediate host and that some arthropod which is used by the armadillo as food acts as primary host.

The type of this genus, *Oncicola oncicola,* is found in South America where it is a normal parasite of *Felis onca* and *F. jaguarundi.* Travassos (1917: 50) has empirically stated that the eggs of *O. oncicola* are ingested by the armadillo (Tatus sp?) and that the larvae freed in the digestive tract penetrate the wall and become encysted in the connective tissue and muscles. Until a direct infestation of the vertebrate has been actually observed it is not safe to assume that the armadillo is the primary host of either species of this genus.

Unless the specimens found by Parker (1909) represent several distinct infestations it is difficult to believe that the ulcerations of the buccal membrane "as from abrasions three or four days old" could have been caused by individuals such as I have examined from his original collection. These specimens were fully mature, the female carrying abundant, fully formed embryos. The exact time required for completion of sexual development in the definitive host is not known for many species of Acanthocephala, but in most instances it covers a period of several weeks.

The hard shelled embryos within the body cavity of the mature female vary considerably in size, ranging, for *O. canis,* from 59 to 71μ in length and from 41 to 50μ in diameter. These measurements are considerably less than those given for *O. oncicola* by Travassos (1917). According to that author the embryos of the South Amer-

ican species are 99μ long and 71 to 75μ broad. Larvae from the peritoneum of the Armadillo are usually 4 mm. or more in length.

Facts are not available to make it possible to pass final judgment upon the prediction of Parker that the coyote may be a usual definitive host of this parasite. No one has ever published a report of having found Acanthocephala in the coyote. Very little work has been done to ascertain the effect of acanthocephalan parasites upon the host. However, the experiments of Grassi and Calandruccio (1888: 524) demonstrate that Acanthocephala when present in numbers cause the host to experience great pain. Calandruccio ingested a considerable number of the larvae of *Moniliformis moniliformis*. In 19 days he was attacked by acute pains accompanied by violent ringing of the ears and of the entire head. In this instance it took five weeks for the larvae to reach sexual maturity so that eggs were recovered from the feces of the patient. It is not at all impossible that pains such as those described by Calandruccio might drive a dog or a coyote "mad."

Literature Cited

Grassi, B., and Calandruccio, S. 1888.—Ueber einen Echinorhynchus welcher auch im Menschen parasitirt und dessen Zwischenwirth ein Blaps ist. Centralbl. Bakt. Parasit., 3: 521-525.

Hall, M. C., and Wigdor, M. 1918. — Notes on the Acanthocephalid and Arthropod Parasites of the Dog in North America. Jour. Amer. Vet. Med. Assoc., n. s., 6: 493-500.

Kaupp, B. F. 1909.—Echinorhynchus canis. Amer. Vet. Rev., 35: 154-155.

Parker, J. W. 1909.—Echinorhynchus canis. Amer. Vet. Rev., 35: 702-704.

Porta, A. 1914.—Acantocefali nuovi e note sinonimiche. Zool. Anz., 44: 483-485.

Travassos, L. 1917.—Contribuicoes para o conhecimento da fauna helmintolojica brazileira. VI. Revisao acantocefalos brazileiros. Parte I. Fam. Gigantorhynchidae Hamann, 1892. Mem. Inst. Oswaldo Cruz, 9: 5-62.

Ward, H. B. 1897.—Animal Parasites of Nebraska. Report of the Zoologist. Ann. Rep. Nebr. Bd. Agr. for 1896, pp. 173-189.

SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The thirty-ninth meeting of the society was held January 9, 1920. Messrs. Boeck, Cort, Daubney, Hegner, Root, Scott and Taliaferro were elected active members. Dr. J. E. Guberlet and Dr. G. A. MacCallum were elected American corresponding members and Dr. S. Yokogawa and Dr. S. Yoshida were elected foreign corresponding members.

The following note was presented by Dr. Cobb:

NOTES ON TYLENCHUS PENETRANS AND T. DEVASTATRIX

In shipments of lily-of-the-valley bulbs from Holland, the roots show discolored areas, which on examination prove to be heavily infested with what the authorities in Holland have regarded as *T. pratensis* deMan, but which has been found to be *T. penetrans* Cobb. This pest is so common on these bulbs in Holland that the Dutch authorities have notified American authorities that they can not guarantee freedom from it. German bulbs are similarly affected.

This has raised the question as to the degree of prevalence of the parasite in this country. It has been found on roots of potatoes, cotton, violets, and camphor. Around Washington, D. C., and adjacent states, lily-of-the-valley bulbs are free from this, but harbor 19 other nemas.

In this connection it may be noted that in a shipment of about 15 species of plants from Brazil, there were found in a few ounces of attached soil 76 species of nemas belonging to 30 genera, of which species 75 per cent were new. In soil from the roots of one species of plant from Guatemala there were 23 species of nemas, of which 61 per cent were new. In bamboo from Japan, grown in South Carolina, there were eight species of nemas, of which 75 per cent were new. In dirt ballast from Trieste, Italy, 14 species of nemas were found, 79 per cent of which were new.

Red clover raising in the North-west has been seriously interfered with by the injury resulting from infestations with *Tylenchus dipsaci* Kühn (*T. devastatrix*), probably imported from Holland on hyacinth bulbs. This species also occurs on the onion. In Idaho it has done so much damage that it has been necessary to plow out second-year clover, thus occasioning considerable loss. An investigation shows that the live nemas may occur on the seed even when recleaned. As this species has been worked on by Ritzema Bos, an inquiry was sent to him in regard to the present knowledge of resuscitation of the worm. In reply he states that the eggs and larvae survive after desiccation for varying periods. In some stages the worm will survive after desiccation for 6 days; in others after desiccation for 2½ years. The worms are best revived in a well aerated film of water and may require from a number of hours to two weeks to again show signs of life. This is probably true of many soil nemas, a point of interest in connection with the wide distribution of these worms in commerce. Dr. Cobb stated his belief that the above incidents are a reliable indication of the extent to which nemas are distributed in ordinary commerce.

Dr. Cort presented the following note:

A PECULIAR MODIFICATION OF THE TAIL IN A CERCARIA

A number of theories undertake to explain the morphology of the cercaria tail. It has been surmised that the tail serves as bait for the host animal in cases where the tails resemble annelids belonging to the Tubificadae in their general outline and movement. The tail also serves in locomotion and attachment, and possibly in floating and in some instances forms a cyst for the body of the worm. In a form found in California the interpretation of the tail struc-

ture is very difficult. In the case of a similar form described by Ssinitzin, this writer has suggested that the tail serves to hold the cercaria in place in the host. An examination of the curiously modified tail in the California specimens shows that the tail appendages are connected with the excretory system and that the tail has only a weak attachment to the body, pulling loose very soon after escaping from the redia. In this species the tail could not function as a cyst, as the tail bulb has too small an aperture connecting it with the body. There are two ribbon-like processes of unknown function extending from the back, and a very long process connecting with the excretory system.

Dr. Stiles gave a very interesting talk on his experiences during the war, including helminthological work and other activities.

The fortieth meeting of the society was held February 20, 1920.

Doctor Ransom presented the following note:

INTESTINAL LESIONS IN CALVES DUE TO COOPERIA PUNCTATA

Post mortem examination of calves dying during the late autumn and early winter at the Experiment Station of the U. S. Bureau of Animal Industry at Bethesda, Maryland, disclosed the presence of heavy infestations with several species of nematodes, including lungworms, stomach worms, hookworms and a small trichostrongylid, *Cooperia punctata.* The last-named species occurs in the stomach and intestine and has been found in cattle in Europe as well as in the United States. The adult worms have been reported as occurring in the lumen of the digestive tract, but in this outbreak of disease at Bethesda it was found that they also occurred in the intestinal mucosa of the upper part of the small intestine. In the mucosa they are present in lesions which are visible to the naked eye as small accumulations of white or yellow caseous material. The same type of lesion associated with small worms, which in all probability are the same species, has been reported from calves in Mississippi and Louisiana by veterinarians in those states in verbal communications. Specimens of worms from apparently similar cases have been sent in from Venezuela by Doctor Ribero. In this material the worms are somewhat larger and more robust as a whole and in detail, the spicules attaining a maximum length of 175μ as compared with 150 to 155μ in worms collected in this country and in Europe, but the species is apparently identical. *C. punctata* is commonly associated with *Cooperia oncophora* in the intestinal lumen, the former being most numerous in the upper part of the small intestine and scarce in the lower part, the latter on the other hand being rare in the upper part and more numerous in the lower part of the small intestine. In this connection it may be noted that the young hookworms found in calves at Bethesda occurred in the lower part of the small intestine, whereas the mature worms were most numerous in the upper part of the small intestine. It is also of interest that in a number of cases hookworms were present in the fourth stomach as well as in the small intestine.

Doctor Cort exhibited a specimen of *Taenia saginata* in which one segment showed a genital papilla on each margin, the male tubules being apparently complete on both sides and the female tubules being complete and apparently functional on one side only, the other side presenting only a vaginal rudiment at the margin, which did not connect with the oviduct. The discussion of this tapeworm, a species which abounds in abnormalities, brought out other reports of similar variations and a consideration of some of the species which have been based on abnormal specimens of *T. saginata.*

Doctor Cort called attention to the fact that the Japanese government is sending 80 men each year to other countries for study and investigation, and that among these men this year there are five parasitologists, Yokogawa, Yoshida, Miyagawa, Miyauri and Goto, of whom the first two are in the United States.

Doctor Yokogawa exhibited some drawings of a Heligmosomum from the rat, *Epimys norvegicus,* in Baltimore. The species is of special interest on account of a marked asymmetry of the bursa. The discussion of this worm brought out a number of points in connection with asymmetry in nematodes and other animals, among others the asymmetry of the head in the louse, Philopterus, and of the bursa in Haemonchus and Bunostomum.

Doctor Pfender gave a discussion of oral infections and the possible relation of amebae to these infections.

Mr. Schwartz presented the following note:

ACTIVE SUBSTANCES IN MACRACANTHORHYNCHUS

Physiologically active substances that are known to occur in parasitic worms may be conveniently grouped under the headings (1) enzymes, (2) hemotoxins, (3) toxic substances which have been designated as leucomains by certain writers, but which are more commonly referred to as teniotoxins, askaron, etc., depending upon the group of worms in which they occur. Tests for the presence of enzymes in *Macracanthorhynchus hirudinaceus* (*-Gigantorhynchus hirudinaceus*) were performed on the fluid which occurs in the general cavity of the worm, on watery extracts of the entire body substance of the worm and on the fraction of the worm that is precipitated by absolute alcohol. The results were identical in each case. Neutral olive oil was not digested by the fluid of Macracanthorhynchus after a week's incubation at 37 to 38° C. Protein material such as coagulated egg albumin and fibrin stained with Congo red, showed evidence of digestion by the fluid Macracanthorhynchus by setting free the stain which colored the solution after 24 hours at 37 to 38° C. The weak proteolytic action of the fluid was observed in an alkaline medium and was completely inhibited in an acid medium. Small shreds of fibrin were not dissolved after being in contact with an alkaline solution of the fluid for a week. Proteolysis by Macracanthorhynchus fluid is therefore a markedly slow process. In contrast to the feeble digestion of proteins, digestion of starch occurred quite rapidly. Starch paste, boiled and unboiled potato starch, were converted into sugar by the fluids of the parasite. Tests for the presence of oxidizing enzymes were negative. Hydrogen peroxide remained intact after the addition of Macracanthorhynchus fluid. A tincture of guaiac was similarly unaffected, even in the presence of a weak solution of hydrogen peroxide.

Concerning the presence of hemotoxins in Macracanthorhynchus, a limited series of experiments have yielded positive results. The fluid from the general cavity of the worms was found to be hemolytic to washed red blood corpuscles of cattle and swine. Dried worm material was powdered and extracted in physiological salt solution. The opalescent supernatant fluid was tested on washed rabbit blood corpuscles with the following results. After one to two hours' incubation the corpuscles became agglutinated but not hemolyzed. Eighteen hours later, during which interval the tubes containing the mixtures of corpuscles and worm fluid were kept at 8° C., hemolysis had occurred. In the presence of normal rabbit blood serum the hemolytic potency of the extract was paralyzed. The potency of the extract was destroyed by boiling. Tests on sheep erythrocytes yielded negative results.

Doctor Cobb exhibited some apparatus useful in the collection of parasites, including (1) screens in which the intersections of the mesh are welded to make an integral construction strong enough to permit of the use of large screens, without the resultant distortion which occurs in ordinary woven mesh; and (2) a Syracuse watch glass painted black on the bottom and graduated on the bottom inside, to facilitate collection by giving a contrasting background.

The forty-first meeting of the society was held March 20, 1920.

Dr. Hall presented a note on intestinal parasites found in 18 Alaskan foxes from St. George Island; all had ascarids, ten had a very small species of

Mesocestoides, apparently new, one had an undetermined species of Taenia, a very small worm, and one had a number of dipterous larvae, probably from fly-blown flesh. None of the foxes had hookworms, which are a serious pest in some places. In comment, Dr. Cort noted the presence of a small Mesocestoides in the mouse in Colorado and a Mesocestoides from the coyote in California.

Dr. Hall also presented a short note on anthelmintics, covering two points: 1. That where oil of chenopodium is followed after an hour or longer by a purgative, Epsom salts may be superior to castor oil in that salts usually cause purgation more rapidly than does castor oil, but that where purgatives are given with the oil of chenopodium, castor oil is quite satisfactory and seems, in some cases, to have given protection of some sort apart from its purgative action; 2. That tests of anthelmintics on earthworms are only tests of toxicity for the substances tested and that the results are, for the most part, only applicable to earthworms. Tests in vitro even on parasitic worms tell little about the anthelmintic value of the drugs tested. Even the application of results obtained in administering anthelmintics to remove worms from one species of animal must be made with reserve to other species which are infested with closely related worms.

Dr. Ransom presented the following note:

THE OCCURRENCE OF ONCOCERCA IN CATTLE IN THE UNITED STATES

About ten species of Oncocerca have been reported from man and the domesticated animals. The life history of none of these has been worked out. *O. reticulata* occurs in the suspensory ligaments, flexor tendons and other connective tissues of the legs of the horse, and *O. cervicalis* in the cervical ligament of the same host. *O. volvulus* occurs in man in Africa, in nodules in the subcutaneous connective tissue usually on the body, rarely on the hand. *O. caecutiens* Brumpt, 1919, which occurs as a parasite of man on the west coast of Guatemala, is very similar to *O. volvulus,* but is located practically always on the head. It causes a disease known as coast erysipelas. Robles has operated on over a thousand cases with almost invariably prompt relief to the patient following the removal of the parasites. *O. gibsoni* occurs in cattle in Australia, usually on the brisket, and is of considerable importance in meat inspection with especial reference to the export beef trade. This species is quite certainly absent from the United States.

In the United States, either *O. reticulata* or *O. cervicalis,* and probably both, occur in the horse, and *O. lienalis* (Stiles, 1892) in the gastro-splenic ligament of cattle, the last named being common and widely distributed. There has also been found in cattle in this country, a species which occurs in a superficial position in the ligamentum nuchae and in the ligaments of the legs, especially at the knees. This form seems rather common in cattle slaughtered at Chicago. The lesions produced are slight, consisting of small local edemas and discolorations in the connective tissue and sometimes calcareous concretions in cases in which the parasites have degenerated. This parasite is of comparatively little importance in meat inspection; the species remains to be determined.

Dr. Simon reported the occurrence of an Oxyuris in the Canadian porcupine. The discussion developed the fact that this worm is probably *O. evoluta* which was described from the porcupine.

Mr. Schwartz presented the following note:

ANTIBODY PRODUCTION BY ASCARIDS

According to certain investigators, tests by the Abderhalden reaction are positive for animals known to be infested and negative for noninfested animals, but too much stress can not be laid on this fact in view of what has been

learned of this reaction. Tests by complement fixation reaction on 17 hogs, showed 16 negative and 1 positive. Fecal examinations of the same animals showed 10 uninfested and 7 infested. The positive reactor showed infestation. In this connection it should be noted that eosinophilia is infrequent in ascariasis. This fact and the failure of infested animals to show a positive reaction by complement fixation indicate that absorption of substances derived from or elaborated by ascarids is inconstant, the conditions determining the absorption being unknown. When rabbits are immunized with material from *Ascaris lumbricoides,* they show a positive complement fixation, indicating the production of antibodies. The reaction is not strictly specific, since it can be worked by antigen prepared from *Ascaris equorum.* The antigen must be kept cool and used fresh, which indicates that it is very unstable.

Dr. Ransom called attention to serum sickness occurring in his own case from the entrance of a minute amount of ascarid fluid into an abrasion on the hand. There was a prompt local reaction, rapidly extending, followed by general urticaria, slight dyspnea, pulse of 150, swelling of arms and face, and an increase of polymorphonuclears in an hour to 80 per cent.

Dr. Hassall discussed the question as to what constitutes a legitimate place of publication for scientific names. This matter is of importance since the application of the law of priority depends largely on it. The place of publication should be as legitimate as the name published.

Mr. Chapin exhibited some specimens of wasps parasitized by members of the Stylopidae (or Strepsiptera). Among these forms, the female is a permanent parasite of the wasp's abdomen. Wasps become infected as larvae, the female Stylops remaining on the wasp, while the male leaves in the spring. Wasps attack and destroy these males whenever possible and it is advisable in breeding experiments to have a partitioned cage with a screen through which the Stylops can pass and the wasp can not. The male Stylops seeks out the unfertilized female and copulates on the wasp, the female later swelling to form a bag of eggs. The resultant larvae swarm out and collect on flowers or other places where they can attach to suitable host insects. These insects carry the larval parasites to their nests, where they feed on the cell food and mature. The parasitized wasp shows pronounced changes as a result of the infestation.

Dr. Hegner presented a note on "Measurement of trypanosomes of the newt, *Diemyctylus viridescens*" (To be published in THE JOURNAL).

Dr. Scott presented the following note:

INSECTS AS POSSIBLE HOSTS OF SARCOCYSTIS TENELLA

In view of the fact that a connection had been suggested by Darling between the Cnidosporidia of the insects and the mammalian sarcocysts, a number of experiments with lambs were carried out in Wyoming to obtain information on this point. Lambs were fed numerous insects belonging to various orders and exposed in other ways to possible infection by insects. Check animals were protected from such exposure. In all cases the experiments, which were carried on for four years, gave no evidence in support of the idea that sarcosporidiosis could be associated with insects, and by raising lambs which became infected in a screen cage free from insects the hypothesis was proved untenable.

The forty-second meeting of the society was held May 8, 1920.

Dr. Cobb presented a note on "A newly discovered parasitic nematode (*Tylenchus mahogani,* n. sp.), connected with a disease of the mahogany trees" (published in THE JOURNAL).

He also discussed "The transference of Mononchs from place to place for economic purposes" (published in *Science*).

Dr. Cobb summarized a paper to be published in Nematology, dealing with the morphology of 120 new genera of free-living nematodes.

Dr. Scott presented the following note:

THE OVER-WINTERING OF THE HOUSE FLY IN WYOMING

At Laramie, Wyoming, situated at an elevation of 7,000 feet, the summers are short and the winters long and often severe. To ascertain the possibility of fly larvae or pupae over-wintering under these conditions, a feed rack in which flies were breeding abundantly in the fall was enclosed in a fly-proof cage and examined from time to time during the winter and in the spring. No flies emerged in this cage. It therefore appears that flies can not survive outdoor winter temperature in Laramie while in their larval or pupal stages. It is possible that through trains bring in flies in summer. In the discussion a general belief appeared that the warmer parts of the Southern United States probably acted as a reservoir for a fly supply during the winter and that a large number of flies must be carried North by through trains in spring and summer. Captain Daubney noted that in Mesopotamia flies disappeared during the middle of the summer, apparently owing to the intense heat. He also noted that in one case where flies became very abundant an examination developed the fact that they were breeding in enormous numbers in the horse manure which was being burned. This manure was burned on an elevated screen, after being mixed with straw, and although the manure was burning at the top, the lower layer was swarming with maggots of the so-called "camp fly" (*Musca humilis*).

Dr. Hall presented the following note:

APPARENT ATROPHY OF SPICULES ASSOCIATED WITH INCREASINGLY CLOSE AND PERMANENT UNION OF THE MALE AND FEMALE SYNGAMUS

According to the descriptions of Muehlig and Feuereisen, in *S. bronchialis* of water fowl the union of male and female is not as close or permanent in nature as in *S. trachealis;* the worms are sometimes found separated and it is possible to detach the male from the female without damaging the specimen. In the common tapeworm of poultry, *S. trachealis,* the union is more intimate and permanent; except in the case of young worms in the lungs, males and females are always found attached and it is impossible to pull the worms apart without damaging them. The bursa in this species is small and the spicules are from 60 to 140µ long; appear to be rudimentary. In the Y-worm of cattle, *S. laryngeus,* the union of the male and female is very intimate and the worms can only be detached by tearing one or the other. The bursa is very short and very thick, and the recent work of Sheather and Shilston shows that the bursa of the male is attached to the circumvulvar region of the female by the interlocking of a number of villous projections from the female inserted into crypts in the ventral surface of the bursa. No evidence of a spicule is found even in sections.

The foregoing suggests that with the development of the permanent copula there has been a strengthening of the bursal attachment and, apparently, a simultaneous atrophy of the spicules, terminating in their disappearance in *S. laryngeus* where the bursal attachment is unusually intimate. In comment, Dr. Cobb stated that an increase in the size of the bursa in species of Rhabditis is associated with a diminution in the size of the spicules.

Mr. Schwartz presented the following note:

EFFECTS OF X-RAYS ON TRICHINAE

Encysted trichinae are highly resistant to X-ray radiation. Heavy dosages exert a selective injurious action on the sex cells of the parasites. Twelve days after feeding trichinous meat which had been exposed to heavy dosages

of X-rays adult worms were found in the intestines of rats. The worms showed atrophied gonads, and in the females the receptaculum seminis contained no spermatozoa. After very heavy dosages of X-rays the worms died in the intestines of the rat without reaching sexual maturity. Attention was called to Tyzzer's experiments on the effects of radium radiation on trichinae. In discussion Dr. Cobb reported that he had tested the effects of X-rays on the gall nematode, *Herterodera radicicola.* In about a dozen tests with various exposures no effect on the nematodes was noted and the galls developed normally.

Dr. Lyon noted the effect of the X-rays in destroying spermatozoa without destroying the testicular secretions. He also called attention to the delayed production of cancer from X-rays in individuals where the cancer developed years after exposures.

The forty-third meeting of the society was held May 29, 1920. Dr. Stiles presented the following note:

RECENT INVESTIGATIONS ON EXCRETA DISPOSAL

The factors involved are those of expense and labor. With this in mind, the Nasik method was tested, this being in use in parts of India. A trench is used and the excreta covered with street sweepings; it has been claimed that these trenches are inoffensive and do not breed flies, and that the excreta are available as manure in a year. The availability of sawdust led to testing this and it was found excellent. Since burying excreta brought them closer to the ground water and removed the surface soil with its bacteria, the excreta were left on the ground surrounded by a box-like container of sawdust and were covered with sawdust. There was no odor, the sawdust would not wash away or blow away and would not burn. This method is only applicable where there is plenty of sawdust, but this is the case in the pine woods region through a large part of the rural section where present conditions are insanitary. It dries on top and hookworm larvae can not come to the surface through it; they probably die in a year. Amebae may live up to about 52 days, possibly longer. Allowing a 4-inch range of capillarity, about 6 inches of sawdust will be sufficient cover. Flies will develop from eggs and larvae in the manure and various birds will eat large numbers of the flies as they emerge. The fly problem is not entirely solved as yet.

Tests with wells and pits show that the transport of bacteria through soil is a question of ground water. Bacteria do not travel through thoroughly dry soil; they apparently travel large distances very rapidly with ground water. Contrary to what has been stated, ground water contains ciliates and flagellates.

Capt. Daubney presented a note on the lungworms, *Dictyocaulus filaria* and *D. viviparus,* reviewing his own work on the life history. Artificial infection of experimental animals was accomplished first by Romanovitch and Slavine and later by Guberlet. Daubney also succeeded in infecting sheep with *D. filaria* and exhibited figures illustrating the stages in the life history. He called attention to the confusion of the larvae of Dictyocaulus with those of Synthetocaulus by some writers. He also noted that the larvae are not as resistant to actual desiccation as some have claimed, and where there is a trace of moisture worms may survive for much longer periods. The larvae ascend a moist surface in dampness or diffused light and descend when exposed to direct sunlight.

Capt. Daubney also read a summary of a study of the lesions due to various species of lungworms, noting that the lesions were different in the case of Synthetocaulus from those in the case of Dictyocaulus.

Mr. Chapin reported the occurrence of encysted *Gongylonema scutatum* in *Aphodius rubeolus,* a new host for the larvae of this worm.

Dr. Scott presented a note in regard to the transmission of swamp fever by means of the nasal secretions. The secretions were taken from infected horses and injected subcutaneously or injected into the nostrils. In both cases the experiment animals developed the disease, thereby demonstrating this method of transfer for the first time. It is possible that insects may carry these secretions from the nostrils of one animal to another.

The forty-fourth meeting was a dinner celebrating the decennium of the Society.

MAURICE C. HALL, Secretary.

NEW HUMAN PARASITES

Necator argentinus Parodi, 1920, is described from Argentine and southern Brazil (Sem. med., Buenos Aires, no. 6, 1920). Langeron (Bull. soc. path. exot., 13:539) discusses the significance of the cervical papillae in the Ancylostomes and shows that they are not of diagnostic value. He concludes that the Argentine species is probably the same as *Necator americanus* Stiles.

Entamoeba macrohyalina Tibaldi, 1920, was found in the crypts of the tonsils of two young persons in Italy. It measures 24 to 40μ in diameter with a nucleus measuring 3 to 6μ in diameter, and thus tends to be considerably larger than *E. gingivalis* which is of common occurrence in the mouth and may also be found in the tonsils. It differs conspicuously from *E. gingivalis* in the character of its ectoplasm which forms a broad homogeneous zone of a pale opal tint when stained with Giemsa. There is a well developed contractile vacuole evident in the living organism. The nucleus is without a definite membrane and there is no karyosome. The nuclear chromatin abundant, and in the resting condition is disposed in little clumps closely pressed together in the peripheral portion of the nucleus, leaving in the center a small clear triangular or polyhedral area. (Ann. d'Igiene. 30:613-620, 1 pl., figs. 1-12, Oct., 1920.)

BOOK REVIEWS

Die Tierischen Parasiten des Menschen, die von ihnen hervorgerufenen Erkrankungen und ihre Heilung. By Max Braun and Otto Seifert. II Teil: Klinik und Therapie der Tierschen Parasiten des Menschen. By Doctor Otto Seifert. Pp. 506. Leipzig: Verlag von Curt Kabitzsch, 1920.

The first part of this well-known text appeared just before the opening of the war and was reviewed at length in the Journal (2:201). The second part, covering the clinical-therapeutic section, has only just appeared. The delay permitted the complete reorganization of the text and the introduction of material from the period of the war so that the book contains much not yet available in any other text. There is, of course, no necessary and intimate connection between this part and the section that appeared earlier under the immediate authorship of Professor Braun, but by virtue of a common origin and general title one might expect an agreement in formal matters at least. The fact is, however, that the names employed in this part to designate the parasites are noticeably different from those employed by Braun, even in some cases being conspicuously unusual, antiquated, and erroneous. This is unfortunately calculated to confuse most students and will surely lead astray those without considerable technical knowledge in this exact field.

The author handles his topics in a fashion not found in any other work, emphasizing symptomatology, treatment, drugs, dosage and other practical medical aspects that are peculiarly significant for the practicing physician and make the volume indispensable. Reference to the sources from which material is taken are introduced in such abundance that fifteen to twenty-five percent of every page is taken up by the brief footnotes containing the references. The bulk of the material is German of course and American cases are not very fully, or carefully, listed; but other nations are equally passed over and perhaps the cases listed, which are evidently selected, give adequate illustrations of types as well as methods of treatment and results. The author cites often a review or abstract of an article rather than the original, his references being confined to relatively few journals. This has the advantage of making it possible to consult the article readily but is open to some objection by virtue of greater chance of error and confusion. The great medical journals in Germany and England are most often cited; those of France and the United States come next and all others are infrequently referred to. Thus while citations are abundant and representative, the treatment of the topics is not exhaustive though more extensive than in any other work yet published.

The arrangement of the material seems at times somewhat peculiar and difficult to explain. Thus in the general section on Cestodes many pages are taken up by a discussion of hookworm anemia, of ascaris symptoms and toxins, of whipworm anemia, of pinworm poisons, and of verminous appendicitis. This material is of special value and deserves greater emphasis. Since no general discussion appears under the heading Nematodes, it is possible that this material really represents a misplaced section which by chance was printed under the wrong heading. A parallel case occurs under the heading Myiasis externa where at the close several pages are devoted to organisms surely not in any sense flies, such as the earworm, various Myriapods, Artemisia, and even a Milleporidian coral that produces dermatitis.

The volume has a good author's index but the topical index is weak, as important a parasite as the trichina not being referred under any heading. But these are minor defects and do not conceal the high value of the work. In its first edition Professor Seifert's section represented a new departure in the literature of parasitology. In this new edition the book is larger, better, and even more indispensable than before.

PARASITES AND PARASITOSIS OF THE DOMESTIC ANIMALS. The Zoology and Control of the Animal Parasites and the Pathogenesis and Treatment of Parasitic Diseases. By B. M. Underhill. Cloth. Pp. 379. New York: The Macmillan Company, 1920.

In this text the author presents the subject from the standpoint of a veterinarian. He devotes two chapters to general questions concerning parasitism, eleven to arthropods, twelve to worms, and two to protozoan parasites. This represents perhaps the needs of veterinary practice but is inadequate surely to portray present knowledge of the field.

As the only presentation of the subject in English from a veterinarian, the work is valuable; it indicates what topics appear important in veterinary science, and what aspects of those topics deserve emphasis. The book also contains much that is not found in any recent treatise on parasitology and thereby will command for itself a place in the literature of the subject. It is unfortunate that it displays in some respects a lack of finish that detracts greatly from the effect. The paper is so thick that the book appears padded, the typography is not at all attractive and some pages are very poorly set. Are we to attribute to the war these shortcomings on the part of publishers who ordinarily set and maintain high standards?

Those figures which are copied from the United States Bureau of Entomology are mostly admirable but a few (p. 61) are unnecessarily large and coarse. Some other figures (p. 158) are so poorly copied as to be a reflection on the source and among the original drawings too many are rough and unattractive or even mere caricatures. This fault, which is marked in many recent works, obtrudes itself on the attention and prevents the excellencies of the text from receiving due acclaim.

NOTES

The International Health Board of the Rockefeller Foundation has recently approved a plan for a cooperative investigation on the biology of hookworm larvae in the soil to be carried out by the Department of Zoology of the School of Hygiene and Public Health of the Johns Hopkins University. The investigations will be carried on in Trinidad in connection with the International Health Board's hookworm campaign which is under the direction of Dr. G. C. Payne. The expedition will start about May 1st and will be gone about four months. It will be under the direction of Dr. W. W. Cort of Johns Hopkins University, and associated with him Dr. J. E. Ackert of Kansas Agricultural College and Mr. D. L. Augustine. It is planned to center the researches around the question of the infectivity of the soil and to study the various phases of the life of the hookworm larvae in the soil which relate to this problem.

The Typhus Research Commission of the League of Red Cross Societies to Poland has printed a very important preliminary report of their work (Int. Jour. Pub. Health, 1:211). They found the *Rickettsia prowazeki* of da Rocha Lima constantly in lice fed on typhus patients and also in the vascular lesions of experimental animals infected with typhus. These lesions are thoroughly characteristic of the disease.

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MEASUREMENTS OF *TRYPANOSOMA DIEMYCTYLI* FROM DIFFERENT HOSTS AND THEIR RELATION TO SPECIFIC IDENTIFICATION, HEREDITY AND ENVIRONMENT

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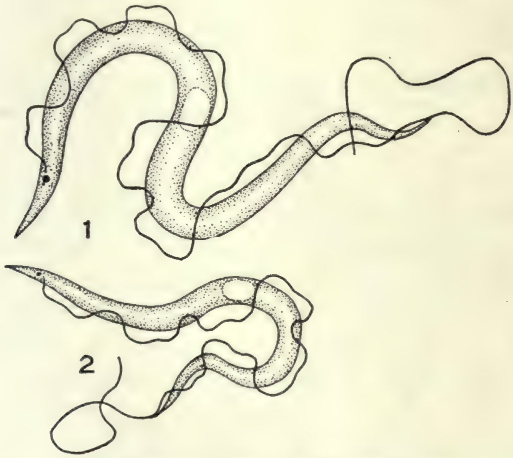
INTRODUCTION

The problems involved in studies of strains of trypanosomes are both scientific and practical. Considerable time and effort have been devoted to attempts to find size differences between human trypanosomes of supposedly different species. Investigators have endeavored also by means of measurements to establish the identity of human trypanosomes with those of certain lower animals and thus to locate animal reservoirs. Furthermore, the data resulting from these researches throw some light upon the possibility of the existence of heritably diverse strains within a species, and upon the effects of the environment, *i. e.* the blood stream, of different species of hosts and of different hosts of the same species on a single strain. It is this last problem that is considered in the following pages.

Trypanosoma diemyctyli (Figs. 1 and 2) was found by Tobey in 1906 to be present in all of a number of newts purchased in an animal store in Boston. During the spring months of 1919 the writer examined the blood of six different species of salamanders. In none of these were any organisms found except in *Diemyctylus viridescens*. All of seventy-eight aquatic specimens of these were infected and two out of seven land specimens. The negative animals were 46 specimens of *Necturus maculosus,* six of *Plethodon glutinosus,* five of *P. cinereus,* 11 of *Desmognathus fusca,* and six of *Spelerpes bilineatus.*

The difference between the aquatic and land forms of *Diemyctylus viridescens* as regards infection with trypanosomes presents an interesting problem. The life cycle of these amphibia includes a year in the water, then a second year on land, and finally a return to the water for mating in the third year. Evidently while on land the infection with the trypanosomes is much decreased. The small numbers of

organisms found in these terrestrial specimens may be due to the absence on land of the transmitting agent, which is unknown. The universal abundance of the trypanosomes in the aquatic specimens may be due to their continued inoculation with young stages that have developed in the intermediate host; and the lesser numbers in the land forms, to a gradual dying out of older trypanosomes. It is of interest in this connection to note that of 34 "land" frogs examined during the spring and summer of 1919 only two were infected with trypanosomes whereas of 41 "water" frogs 28 were infected.



Explanation of Figures

Fig. 1.—Typical specimen of *Trypanosoma diemyctyli* from newt 19. × 1600.
Fig. 2.—Typical specimen of *Trypanosoma diemyctyli* from newt 15. × 1600.

METHODS OF MEASURING TRYPANOSOMES

In any investigation involving measurements the accuracy of the results depends primarily on the accuracy of the measurements. Trypanosomes are difficult to measure precisely, since their bodies are almost always thrown into curves when fixed. This is especially true of long slender forms. Several methods of obtaining accurate measurements have been employed by investigators.

The method adopted by Bruce, Hamerton and Bateman in 1909 was to draw an outline of each specimen with a camera lucida at a magnification of 2000 diameters "and then to measure along the middle line of the body by means of a pair of fine compasses, the points of which are separated 2 mm. Each step the compass takes is therefore equal to 1 micron."

A modification of this method was employed by Stephens and Fantham (1912). They projected the trypanosomes on a screen with

a microprojection apparatus and then traced their outlines with a sharp pencil. A magnification of 2500 diameters was adopted. The drawings were then measured by placing over them semitransparent tracing paper on which a straight line was drawn in ink. One end of the ink line was placed on one end of the drawing and rotated whenever the axis of the trypanosome curved. When the end of the drawing was reached the distance was measured with a millimeter scale.

The method used by the writer seems more desirable than those described above. The trypanosomes were projected with a camera lucida upon a drawing card at a magnification of 1600 diameters. The anterior and posterior ends and kinetonucleus were then indicated with a dot; the width of the body at the nucleus was recorded by two short parallel lines; and the nucleus was drawn. A single line was then drawn down the center of the body from the posterior end to the anterior end. With a chartometer or "map measurer" the distances were easily and accurately obtained.

INVESTIGATIONS INVOLVING TRYPANOSOME MEASUREMENTS

Bruce and his colleagues have measured thousands of trypanosomes of various species in their endeavor to distinguish by size characteristics between the species pathogenic in man and those that occur in the lower animals. The organisms measured were derived from various strains and were taken from a number of species of both wild and laboratory animals. Bruce finally decided that this method of specific identification could not be depended on.

Data regarding the effects of different hosts on the size of the specimens have been provided by various investigators. Thus Laveran and Mesnil (1912) noted a difference between the length of specimens of *T. brucei* grown in the horse and those grown in rodents. Duke (1912) has suggested that strains of numerous varieties exist among trypanosomes of any species, and that alterations in the morphology of a strain may follow continued passage through laboratory animals.

It seems probable from the work of Miss Robertson (1912) on *T. gambiense* that one difficulty in biometric studies of such trypanosomes, is the presence of an endogenous cycle in mammals which results in changes in the types at intervals that cannot be determined by the date of infection. Even if diversities in size were noted in specimens from different hosts the results would not be conclusive. It seems, therefore, that measurements of these polymorphic species are of doubtful value and that better results may be expected when monomorphic forms are studied.

Pearson (1914) has made a biometrical study of many of the measurements published by other investigators and concludes that actual statistical analysis does not in any way confirm the bulk of the

conclusions reached by Sir David Bruce and his collaborators. He points out the fact that the data available do not provide material for an analysis of the relative influence of the various environmental factors and hence one cannot determine whether divergences indicate different strains or merely modifications due to different environments.

Most of the quantitative studies of trypanosomes deal with attempts to secure data that will provide means of specific diagnosis. Those data that might furnish evidence of diversities due to the character of the host in which the specimens were grown are of doubtful value because most of the species studied have been dimorphic or polymorphic and hence have exhibited great variations even from a single host. Furthermore, many of the strains measured had received dissimilar treatment; some were taken directly from wild animals, whereas others had been passed through series of laboratory animals of different species during periods of varying length. This treatment may have had an influence on the morphological characteristics of the strains used. The available measurements of monomorphic trypanosomes do not exhibit variations that make possible any definite conclusion as regards diversities when grown in different hosts.

A review of the literature, especially the paper by Pearson, emphasizes the importance of more careful studies of the relations between trypanosomes and their environment represented by different species of hosts and by different individuals of one host species. It is possible to isolate single trypanosomes and to obtain in one host animal a supply of specimens that can be compared with the descendants of other single specimens in host animals of the same or other species. Work of this character is now in progress in this laboratory.

TRYPANOSOMA DIEMYCTYLI FROM DIFFERENT HOSTS

Measurements were made of 100 specimens of *T. diemyctyli* that were taken at random, ten from each of ten individuals of *Diemyctylus viridescens*. Some of these measurements are presented in Tables 1 and 2. The preparations were all made on the same day and stained with Wright's stain. No selection was made either of the newts from which the trypanosomes were obtained or of the trypanosomes on the slides. The first ten trypanosomes that were found in the preparation from each newt were drawn at a magnification of 1,600 diameters and then measured with a map measurer.

Table 1 includes the variations in the distances from the anterior end of the body to the center of the nucleus, from the center of the nucleus to the kinetonucleus,* and from the kinetonucleus to the posterior end. The width of the body at the point where the nucleus is

* By the kinetonucleus is meant the body called by the French centrosome, by the Germans blepharoplast, and by certain Americans parabasal.

situated is also given as well as average distances. The data are arranged in a series with those of the largest set of ten trypanosomes at the top of the table and those of the smallest set of ten at the bottom. The final averages differ somewhat from those given by Tobey. Tobey's specimens were from 45 to 50μ in length with a flagellum 24μ long. My specimens ranged from 42.5 to 75.3μ in length with a flagellum 32μ long. The measurements of the trypanosomes from newts 19 and 15 are particularly interesting. The data show that in *every one* of the trypanosomes from newt 19 the distance from the anterior end to the center of the nucleus is considerably greater than it is in *any* of the specimens from newt 15. This distance in newt 19 ranges from 31 to 44μ, whereas in newt 15 it ranges from 20 to 23μ. The average in the ten specimens from newt 19 is 36.3μ and in newt 15, 21.4μ, a difference of over 50 per cent. The differences between the distances from the center of the nucleus to the kinetonucleus are similar but not so great, those of the trypanosomes from newt 19 being only 25 per cent. greater than from newt 15. The distances from kinetonucleus to posterior end show differences averaging over 50 per cent., and the average difference in total length exclusive of the flagellum is approximately 50 per cent.

TABLE 1.—Variation in Length and Width of 100 Specimens of *T. diemyctyli* Taken at Random, Ten from Each of Ten Newts, and the Average Length and Width of the Same Groups of Ten. All Measurements in Microns

Number of Newt	Anterior End of Body to Center of Nucleus		Center of Nucleus to Kinetonucleus		Kinetonucleus to Posterior End		Total Length Exclusive of Flagellum		Width of Body at Nucleus	
	Variation	Average	Variation	Average	Variation	Average	Variation	Average	Variation	Average
19	31–44	36.3	23–32	25.6	5.6–6.9	6.1	61.6–75.3	68.0	3.1–3.8	3.3
20	32–42	35.5	23–29	26.1	5.0–6.9	5.9	58.6–72.9	66.5	2.5–4.4	3.3
14	28–33	30.5	26–29	27.7	3.1–5.6	4.3	59.4–66.6	62.5	2.5–3.1	2.9
18	23–36	27.9	22–27	23.9	3.8–5.0	4.3	50.8–65.4	56.1	2.5–3.8	2.9
16	22–31	27.2	20–30	23.9	3.1–5.6	3.9	45.1–65.4	55.0	2.5–3.1	2.6
10	23–31	26.4	21–26	23.9	3.8–4.4	4.3	48.8–60.0	54.6	3.1–4.4	3.4
17	21–27	24.1	22–25	24.0	3.1–3.8	3.5	48.1–55.8	51.6	2.5–3.1	2.7
12	23–29	25.1	21–25	22.9	2.5–3.8	3.3	38.1–57.1	51.3	2.5–3.8	3.0
9	19–29	24.4	18–27	21.8	3.1–4.4	3.6	45.0–60.3	49.7	1.9–3.1	2.6
15	29–23	21.4	19–22	20.6	2.2–3.4	2.7	42.5–48.4	44.7	1.9–2.5	2.2

The striking fact brought out by a comparison of these data is the large and constant difference in length between the two sets of trypanosomes taken from two different hosts of the same species. That this difference in length is not due to methods of preparation causing the elongation of one set and the contraction of the other is evident when a comparison is made of the diameters of the body in the region of the nucleus. The long specimens from newt 19 were also thicker than the short specimens from newt 15, the former averaging 3.3μ in diameter, and the latter only 2.2μ. The trypanosomes in newt 19 were therefore uniformly larger in all dimensions than those in newt 15.

Further study of Table 1 shows that the sets of 10 trypanosomes from each of the 10 newts were comparatively constant in their measurements. On the whole, the longest specimens are also the thickest, and length and width decrease together as one proceeds down the table.

In every set the average distance from the anterior end to the center of the nucleus is greater than that from the center of the nucleus to the kinetonucleus. When these distances are compared in the different sets, however, considerable variation becomes evident. For example, in specimens from newt 19 the difference between these two distances averages 10.7μ whereas in those from newt 17 the average difference is only 0.1μ and in those from newt 15 only 0.8μ. These distances are more uniform also in trypanosomes from newt 15 than in those from the other newts. The variations in the distances from the kinetonucleus to the posterior end were slight in the trypanosomes of each set, but the average distance is greatest in those of the longest set and becomes gradually less as the total length decreases.

After the work just described was completed it seemed desirable to obtain a more accurate measure of the differences between the trypanosomes in newts 19 and 15. Ninety more specimens from each newt were therefore measured by my assistant and the results are indicated in Table 2; this gives the averages of the various distances for the first ten trypanosomes measured from newts 19 and 15, for the succeeding ninety and for the entire one hundred. It is interesting to note that the averages for the first ten are very nearly the same as for the succeeding ninety. This indicates that the data obtained by measuring ten specimens from each newt as presented in Table 1 give fairly accurate averages.

Among the specimens from newt 15 six were found that were very much larger than any of the others. Measurements of these six are given in Table 2 and were omitted from those used for getting the averages of the 100 specimens given in this table. These six specimens resemble very closely those taken from newt 19 and apparently represent a type differing widely from the other more abundant trypanosomes from newt 15. Several explanations suggest themselves to account for the diversity between these two types found in a single host. Most probably there are here two size races of one species or there may be two distinct species living in a single host. The two types may possibly represent sexual stages of one species and although from what is known of the life cycles of other blood-inhabiting protozoa one would expect to find the sexual stages in the invertebrate host, still, as in the malarial organism, gametocytes may be developed in the vertebrate and remain dormant until stimulated to further activity within the invertebrate host. It is also possible that the two

types of trypanosomes from a single host may be due to different stages of growth. A final suggestion is that *T. diemyctyli* is dimorphic.

DISCUSSION

No one has succeeded in classifying satisfactorily the trypanosomes and their allies, a condition due in part to the difficulty of determining morphologic differences of diagnostic value and to the fact that many species are polymorphic. *T. diemyctyli* is a favorable form for study because it is apparently monomorphic. Its life history, however, is unknown. The account Tobey gives of this species and the experience of the writer indicate that types other than the long, slender form do not occur in the blood of the newt, at least at the time of year when the examinations were made (May). No specimens were found that showed any signs of division and hence it seems safe to assume that all of the organisms measured represented "adult" forms.

TABLE 2.—AVERAGE LENGTH AND WIDTH IN MICRONS OF SPECIMENS OF *T. diemyctyli* FROM NEWTS 19 AND 15

	Anterior End of Body to Center of Nucleus	Center of Nucleus to Kineto-nucleus	Kineto-nucleus to Posterior End	Total Length Exclusive of Flagellum	Width of Body at Nucleus
Specimens from newt 19:					
Average of specimens 1-10.....	36.3	25.6	6.1	68.0	3.3
Average of specimens 11-100...	35.1	28.0	6.5	69.6	3.6
Average of specimens 1-100....	35.7	26.8	6.3	68.8	3.5
Specimens from newt 15:					
Average of specimens 1-10.....	21.4	20.6	2.7	44.7	2.2
Average of specimens 11-100...	21.9	21.4	2.3	45.7	2.9
Average of specimens 1-100....	21.7	21.0	2.5	45.2	2.6
Average of 6 largest specimens	33.5	26.3	4.8	64.6	3.5

Two hypotheses suggest themselves to account for the constant diversities in the total length and the length of portions of the trypanosomes from the different individual newts; (1) the observations may deal with pure lines, and (2) the organisms in one newt may be derived from various lines but may have become comparatively uniform in size due to life in one environment. The differences between groups of trypanosomes from different hosts might be accounted for by differences in the environment.

Pure Lines in Protozoa.—It has been shown by many investigators that "wild" specimens of free-living protozoa differ from one another in their heritable characteristics and that the descendants derived by vegetative reproduction from one "wild" individual may be uniformly different from those descended from another "wild" individual. The number of these pure lines that may exist in nature seems almost infinite.

Considerable interest has recently been created by the discovery of different strains of cysts of *Entamoeba histolytica* and *E. coli.* Mathis and Mecier (1916, 1917) recognize cysts of two sizes from *E. histolytica* which they consider indicates a sort of sexual dimorphism. Various strains of cysts as regards size have also been noted in *E. histolytica* by Wenyon and O'Connor (1917), Dobell and Jepps (1917), Matthews (1918), Mackinnon (1918), Smith (1918, 1919) and Kofoid, Kornhauser, and Swezy (1919). The evidence indicates the existence in these parasitic protozoa of heritably diverse races similar to those that have been described in a number of free-living protozoa. What influence environmental factors may have on the size of the cysts can be determined in several ways; for example, single specimens could be isolated from cultures and pure lines obtained from these also in culture. The effects of changes in environment could then be observed by modifying the culture medium or by inoculating specimens from the same pure line into different laboratory animals.

The habitat of the intestinal amoebae resembles that of the trypanosomes in certain respects although it may be more or less varied because of the many different kinds of food taken into the alimentary canal. The composition of the blood differs in different species of animals and to a lesser degree in different individuals of the same species. This means that trypanosomes also are subjected to differences in their environment. No one knows what effect these different environments may have on trypanosomes belonging to the same pure line, but as noted above a method of determining this point is available and is being put to the test in this laboratory.

SUMMARY

(1) Every one of 78 aquatic specimens and 2 of 7 land specimens of the newt, *Diemyctylus viridescens,* collected in Pennsylvania were found to be infected with *Trypanosoma diemyctyli* Tobey. No trypanosomes were found in 72 specimens of 5 other species of salamanders. Inoculation experiments with *T. diemyctyli* on 6 species of salamanders and 2 species of frogs were unsuccessful.

(2) Measurements were made of trypanosomes from 10 newts. These groups of trypanosomes differed from one another in their range of variation in total length exclusive of the flagellum, in the length of portions, and in the width of the body, in the average length of the entire body exclusive of the flagellum, in the average length of portions and in the average width.

(3) Length and width show a positive correlation and on an average the longer the specimen the wider it is.

(4) Of 106 trypanosomes from one newt, 100 were uniformly small and the remaining 6 were much larger, indicating 2 different types in a single host.

(5) The different types of trypanosomes obtained from the different newts are probably races of one species that are heritably diverse in size. They may, however, belong to different species or may be sexual phases of a single species, or may differ because of changes due to the environment.

Literature Cited.

Bruce, Sir David. 1914.—Classification of the African trypanosomes pathogenic to man and domestic animals. Trans. Soc. Trop. Med. and Hyg., 8: 1-22.

Duke, H. L. 1912.—A camel trypanosome with some remarks on the biometric method of diagnosing trypanosomes. Proc. Roy. Soc., B, 85: 563-568.

Pearson, K. 1914.—On the probability that the two independent distributions of frequency are really samples of the same population, etc. Biometrika. 10: 85-143.

Robertson, Muriel. 1912.—Notes on the polymorphism of Trypanosoma gambiense in the blood and its relation to the exogenous cycle in Glossina palpalis. Proc. Roy. Soc., B, 85: 527-539.

Stephens, J. W. W., and Fantham, H. B. 1912.—The measurement of Trypanosoma rhodesiense. Proc. Roy. Soc., B, 85: 223-234.

Tobey, E. N. 1906.—Trypanosomata and trypanosomiasis. Jour. Med. Res., 10: 117-146.

1906 a.—Trypanosomes in the newt. Jour. Med. Res., 10: 147-148.

York, W., and Blacklock, B. 1914.—The differentiation of the more important trypanosomes. Ann. Trop. Med. and Parasit., 8: 1-12.

References to other literature on the specific identification of trypanosomes may be found in the above papers, especially that by Pearson.

A NEW BLOOD FLUKE FROM TURTLES*

HENRY B. WARD

For several years an interesting trematode has been under observation in the laboratory here. It occurs in various species of turtles, and was first discovered in some material shipped in from the south for class work. Peculiar importance attaches to the fact that it is a species inhabiting the circulatory system, and in fact it shows a relationship to the blood-inhabiting flukes of man which has become more clearly evident as the observations have accumulated. Since the material is easily obtained, it will afford perhaps the best opportunity available in this country for the laboratory study of forms adapted to this peculiar environment, so that, despite the incompleteness of the observations, the publication of this note is justified. It is further called for by the fact that several others, who had their attention called to this species, plan to give it a more detailed study than I can make at the present time, and will be glad to have a record of the facts thus far determined in order to utilize them as a basis for further study.

For this very unique species I propose the name *Proparorchis artericola* gen. et spec. nov.

The parasite has been found in several distinct species of turtle from widely separate localities. Thus, according to records of the collection here, it has been met with in *Pseudemys elegans* from Havana, Illinois, in *Malacoclemmys leseuerii* from Newton, Texas, in *Pseudemys scripta* from Raleigh, N. C., and in *Chrysemys marginata* from Fairport, Iowa.

OBSERVATIONS ON LIVING MATERIAL

The general distribution of the parasite in the body of the host is well illustrated by the record of one very careful examination made in May, 1915. The specimen was *Pseudemys scripta*. The circulatory system was first studied and the examination of a large quantity of blood gave only negative results. After ligating veins and arteries, the heart was removed and four flukes found in it. Several large veins were taken out and teased, but no flukes obtained. When, however, the large arteries were subjected to similar treatment, three flukes were taken; one was found plugging up the end of an artery. Both lungs were teased out; one yielded three flukes, the other none. Negative results came from similar handling of the liver. All organs examined

* Contributions from the Zoological Laboratory of the University of Illinois, No. 176.

contained eggs. A large number were present in the brain. They seemed to be more numerous in the lungs and digestive system than in the muscles.

A year later another turtle of the same species was examined with almost identical results. Every precaution was taken to prevent transfer of material from one organ to another or loss of any during the examination. The heart contained four large flukes, the arteries three, and the veins none. One lung yielded three flukes and the other none. The intestine and mesentery were stretched out and fixed in that condition. A methodical examination revealed no flukes in these organs altho eggs were very abundant in the mesenteric vessels.

When the living worms are released from blood vessels into normal salt solution, they often display marked activity and swim about so rapidly that it is difficult to follow them. The method of progression in the fluid resembles that of leeches and is sufficiently powerful to convince the observer that they can probably make progress against the blood stream in the arterial circulation. Their orientation in respect to the direction of the current in the vessels varies.

As has already been noted, they were never found in the venous circulation, but were taken in arteries and in the heart. This location was finally verified by ligating the blood vessels, removing the heart and sectioning it *in toto*. A fluke was found in the ventricle. In this host, also, it was found that eggs were very abundant in the walls of the ventricle and present tho less abundant in the walls of the auricles. The numerical difference was somewhat proportional to the thickness of the muscular tissue in these two regions. The turtle examined in this instance was rather small, and hence young. Yet it was generally infected with eggs in various tissues.

Almost all tissues contain eggs in case the turtle is generally infected but the mesentery and the lungs seem to accumulate the most. In extreme cases these organs are crowded so full that, as can be most easily observed in the case of the mesentery, the eggs serve to outline the course of the arteries. In the mesentery the ova vary widely in color and general appearance. Some are only faintly colored with a clear transparent shell that enables one to determine readily the character of the enclosed embryo. Those at the other extreme are deep brown, almost black, and entirely opaque. None of the shells had opened and apparently none of them escape from the vessels. The mesenteric arteries would thus form a graveyard in which ova would accumulate without achieving for the species their purpose.

It is interesting to compare with this an observation reported to me by one of my students. He examined a small turtle that had died after being kept some time in a laboratory tank and found the lungs filled

with eggs. Many of them were empty shells from which the miracidia had escaped for the lid was open. It is of little value to speculate on the fate of these embryos. It is clear, however, that if at the time of oviposition the flukes resort to the ventricle, which they surely do visit as I have shown, then the ova will be carried in part into the systemic arteries and in part into the pulmonary arteries. The ultimate results seem to be different in the two cases. Eggs have also been obtained from the feces of infected turtles.

The number of parasites found in a single host has never been very large whereas the number of eggs was often very great. This points to the gradual accumulation of the ova in the vessels, perhaps over a considerable period. Some turtles have been examined without finding any of these flukes and yet eggs occurred abundantly in the tissues. In other cases only very young flukes were found and these had not yet begun to produce eggs altho the eggs were numerous in the mesenteric vessels. While some parasites might easily be overlooked, yet these cases indicate that the flukes which had produced the eggs, had died and the young parasites were a later infection.

Some observations were made on the eggs containing living embryos. For this purpose eggs were taken from the feces of *Chrysemys marginata* and treated with dilute solutions of HC1 from 0.02 to 0.1% in strength. The effect on the miracidia was very evidently stimulating, but those that hatched out were killed by the action of the acid immediately after the rupture of the membranes, and some were killed while even yet within the unbroken egg shell. This would seem to indicate extreme sensitiveness to gastric digestion and preclude direct infection of a new host by way of the alimentary system at least.

Eggs placed in hanging drop cultures hatched out in from 4 to 24 hours after being mounted. From these eggs and also from others placed in pure water, the miracidia seemed to emerge sooner than from eggs left in feces in a petri dish. When eggs with mature embryos are broken open in normal (0.75%) salt solution, the miracidia can be studied and later preserved. Free miracidia were found in feces cultures, but all those seen there were dead. Their escape from the shell was not observed. Other eggs in the feces contained apparently fully developed miracidia that had died without hatching out. No light was obtained on the conditions controlling the normal escape of these embryos from the eggs.

The unripe embryo is rather quiet, but shortly before hatching it becomes increasingly active, first by moving parts of the body and then by rotation on its longitudinal axis. This rotation increases in rapidity and is accompanied by pronounced contractions of longitudinal and circular muscles which turn the embryo so that it may assume any posi-

tion within the shell. These violent movements ultimately loosen the cap of the egg shell and tilt it to one side like a lid fastened by a hinge. The miracidium forces its way bit by bit thru the open door which is not large enough to permit its immediate exit, but once free it swims round and round in the free water with relatively great speed and energy. In all observations made here, they lived only a very short time (5 to 10 minutes), becoming rapidly distorted and ceasing all activity thereupon. It is probable accordingly that these observations did not present normal conditions for opening the eggs or for the miracidia afterwards. Of course the conditions may have brought about precocious hatching, but none of the eggs hatched which were held under observation for longer periods.

Miracidia still enclosed in the egg shell measure about 28 to 14μ whereas those free in water or feces are distinctly longer and slenderer, measuring about 30 by 11μ. Within the shell one finds a large oval globule (Fig. 7) slightly greater in dimensions than the embryo. The miracidium has a large black eye spot which always appears irregular, and in favorable circumstances shows the form of contiguous reversed crescents usually designated as X-shaped. Some large gland cells are seen faintly in the living specimen, and its surface is covered with a coating of long cilia which are comparatively thinly distributed. The anterior end carries a cap-like structure which in the free swimming miracidium (Fig. 9) becomes a small bluntly rounded conical papilla. The ducts of the glands open on the summit of this papilla.

STRUCTURE OF THE ADULT PARASITE

The adult worms, which are easily found on careful examination of the mesenteric vessels of infected turtles, are small and conspicuously transparent. In size, they measure from 1.62 by 0.28 to 2.62 by 0.77 mm. In the smallest the ovary was small and no ovum had yet developed but ripe sperm cells were found in testes and vesicle. The body is an elongated oval, or spindle shaped tapering slightly towards sharply rounded ends. The anterior end is more nearly pointed and much more mobile than the posterior. The body is relatively thin, measuring not more than 70 to 80μ in dorso-ventral diameter, and in the preserved specimen is regularly hollowed out a little on the ventral surface both longitudinally and transversely. The margins of the body are noticeably thin and sharp. In the blood vessels the worm appears to be much slenderer and longer than when observed outside the body of the host or in alcoholic specimens. The transparency of the body is due to the relatively slight development of the muscular layers which are represented only by thin sheets of very delicate fibers.

At the anterior end one notes the single sucker present in this species. It is peculiar in form, being a greatly elongated oval with relatively small sub-terminal opening. It projects forward in an unusual fashion, and imparts to the anterior end a characteristic appearance which is rarely met with among trematodes.

The surface of the body is smooth and without spines or scales. None of the small wart-like structures with fine spines have been found in this species which are described by Looss and others for other types of blood-inhabiting flukes. In preserved worms, which are somewhat contracted at the anterior end, the esophagus is slightly sinuous and the inner wall plicated. It has a relatively large lumen and increases in external diameter posteriad. The cavity varies noticeably in width, having one or two wider regions much such as are figured in the Schistosomatidae by Looss (1895, pl. 2, fig. 18). There is no evidence whatever of a pharynx, but near the posterior end (Fig. 5) the wall of the esophagus is conspicuously thickened by an accumulation of what are certainly gland cells. These take a deep stain, and while so irregular in form as to preclude the possibility of interpreting them as a muscular organ, yet superficial examination might lead one to designate this region as a pharynx. It is, however, at the very termination of the esophagus, taking in one-fifth or one-fourth of the entire length of the organ and not separated by any interval whatever from the diverging crura. These gland cells are densely crowded and in this posterior region occur in several layers so that they seem to form an enlargement of the esophageal wall. A thinner layer covers the wall of the esophagus for its entire length. Similar conditions were originally described by Leuckart for Schistosomes and fully verified by Looss. The likeness between Proparorchis and Schistosoma in respect to the esophagus is so complete that it extends even to minute details of structure. Looss (1899:751) reported similar glandular structures in Hapalotrema and denominated them salivary glands. It is thus evident that they are all but universal in blood-inhabiting flukes and indeed will probably be found in species from which they have not yet been recorded. Their development is undoubtedly due to the type of food utilized by these flukes.

The intestinal crura are markedly sinuous in outline and nearly equal in caliber throughout the entire length. They extend to within a short distance of the posterior end and there turn somewhat towards the center, although always remaining distinctly separated from each other. The cells which line them are filled with a dark granular substance, suggesting the origin of this material from the blood of the host (Cf. Looss, 1895, pl. 3, for Schistosomes). The crura diverge almost at right angles from the esophagus, forming a conspicuous cross bar

and an equally conspicuous angle at the side where they turn backwards. Directly opposite the point of junction with the esophagus is a median structure which stains conspicuously and is apparently glandular. It has only in part the same appearance as the crura themselves, and might be regarded as a median diverticulum with a very short lumen. However, the cells are not filled with the dark granules which impart to the intestine its characteristic appearance. The lining of the diverticulum is a very thin membrane and at its base is a mass of amorphous material which resembles in appearance and in staining qualities the inner layer of the esophagus (Fig. 5).

The excretory system is easily seen at the posterior end of the body. It presents the form of a bifid bladder or perhaps of small lateral bladders connected by very short stalks to a common duct, which is equally brief and opens at the median pore. The latter is nearly terminal in location. The lateral bladders are a little shorter than the space between the posterior tip of the body and the end of the intestinal crura. Anteriorly one sees a single longitudinal vessel connected with each bladder. Further details of the system have not been worked out.

The main features of the nervous system are distinct in living specimens and also in toto-preparations. The anterior ganglion spans the esophagus a short distance back of its junction with the oral sucker. The lateral nerves are relatively heavy and can be traced the length of the body. These features are relatively larger and more conspicuous than in most flukes. Here again the conditions recall those in the Schistosomes as reported by Looss (1895).

The most striking feature in this parasite is the peculiar development of the reproductive system. The organs are nearly all confined to the area within the intestinal crura. The testes (Fig. 1, *t*) occupy the major portion of the space anteriorly. They begin a short distance behind the fork of the intestine and extend as a series of irregularly lobed bodies down the median line a distance equal to about one-half of the entire length of the worm. In this group are from six to ten or more irregularly shaped bodies, more or less flattened on the anterior and posterior faces by mutual pressure but deeply lobed on the lateral aspects. In many cases it looks as if the parts were continuous, but sections show well developed limiting membranes separating them. It may be that there is a fixed number of separate parts in this testicular area but the varying stages of contraction in different specimens make it difficult to reach a positive conclusion. Immediately behind the posterior testis is a seminal vesicle (Fig. 3, *sv*) which is elongated, pyriform and connects directly with the cirrus (*c*). No distinct prostate cells were seen and both the cirrus and the cirrus sac are delicate and difficult to detect. The pyriform vesicle and the duct form a nearly

straight passageway from the center of the posterior testis to the common genital pore (*gp*). This opening is located about on the level of the intestine at the left side and ventral.

The ovary (*ov*) is a many-lobed structure in the intracrural area behind the testes; it lies chiefly dorsal. The vesicle and cirrus cross ventrally the left ovarian lobes. The opposite face of the organ is pressed closely against the intestine on the other side of the body. The yolk glands (Fig. 3, *v*) are exceedingly voluminous. They begin about at the end of the esophagus and extend just a little beyond the posterior ends of the intestinal crura. The cells tho not crowded form an almost continuous strip or band which lies below and, to some extent, on both sides of the crura but only in the immediate proximity of those structures, for the central area of the body is entirely without yolk cells. At the end of the esophagus and behind the crura, the cells from the two sides approach and become confluent in the median line. Behind the ovary on the ventral side of the body, the transverse yolk duct joins the two yolk glands and on it in the median line is formed a prominent yolk reservoir (*yr*). There are no reproductive organs behind this limit, except some of the outlying cells of the vitellaria already mentioned.

The ducts of the female system are strikingly simple, and are crowded together in a small triangle between the ovary, the cirrus, and the transverse yolk duct. The relation of the different structures will be apparent from the illustrations (Figs. 2, 3) one of which represents a reconstruction of this area from a series of sections. One can readily identify the various structures. A small expansion on the oviduct near the ovary is seen to be the receptaculum seminis uterinum (*rsu*) which is easily recognized by the considerable mass of sperm cells that it contains. After a brief course dextrad and posteriad the oviduct turns sharply back on itself near the intestine and swings in a crescentic curve to the sexual pore on the opposite side of the body. About at the angle made by this turn, there is given off a short tube which mounts almost directly to the dorsal surface. This is Laurer's canal (*lc*). It is relatively large and open, and contained ripe sperm cells in those specimens which were sectioned. Half way from this point to the genital pore, the canal is slightly expanded, and in this expansion lies in many specimens a single egg. This tube corresponds in position and connections to the long convoluted uterus in most flukes, but instead of carrying a mass of eggs such as is usually found in that organ, it never contains in this form more than a single one. The short stretch which intervenes between this region and the pore is distinctly provided with a muscular layer in the wall (Fig. 4). This region is the metraterm; the egg lies really in the ootype and a true uterus is lacking.

The eggs outside of the worm in the blood-vessels of the mesentery as already noted are in part light colored and semi-transparent, and in part dark brown and almost opaque. The latter seem to be the older eggs. Sets of eggs from different places were measured, and the results are given in the following summary. All measurements are given in microns.

Preparation, No.	Number Measured	Average Length	Average Breadth	Length Max.	Length Min.	Breadth Max.	Breadth Min.
15.54	19	95.9	75.5	105.6	70.4	96.8	61.8
15.71	20	103.4	77.4	123.2	88.0	88.0	70.4
15.71	20	106.5	78.7	124.2	81.0	94.5	64.8
15.72	20	110.4	81.7	121.5	97.2	91.8	75.6
15.72 *l*	12	101.2	82.7	114.4	70.4	96.8	52.8
15.72 *d*	20	95.9	77.0	114.4	79.2	88.0	52.8
General average..........		102.2	78.8				

l = light eggs only; *d* = dark eggs.

Most eggs come within the limits of 88 to 114μ in length and 70 to 88μ in breadth.

When these figures, which are obtained from preserved material, are compared with those giving dimensions of the eggs under other conditions, the results are rather extraordinary. In living specimens eggs from cultures, drawn and measured were 85x68 μ, 97x80 μ, 80.5x73 μ, 85x74 μ, 97x80 μ. In worms which had been preserved, stained and mounted *in toto,* the eggs still contained in the uterus of the female showed dimensions of 75x45 μ and 84x41 μ. The eggs from cultures and especially those still retained in the body of the female are thus smaller than the average of those found free in the blood-vessels of the mesentery. Further, eggs in the body of the worm are clearly oval, whereas those outside are more nearly spherical. Looss (1902:522) noted also that the egg shell may increase in size during the growth of the embryo; this was observed in a blood-inhabiting species the adult of which was not identified.

The eggs found in various organs occasionally show stages in cleavage or much more often two black eye spots indicating that the miracidia are well developed. One encounters also many eggs in which the enclosed embryos are dead and undergoing disintegration. One of my assistants at one time found a mass of eggs in the intestine of a turtle but no evidence was secured on the method by which the eggs escaped from the vascular system or the place at which such escape was made; and the discovery noted may have been based on some sort of accidental transfer of the eggs to the intestinal contents. Nevertheless there is no doubt that eggs occur regularly in the feces of the turtles for they have been collected and studied frequently and those found there contain living embryos.

The data just given on the structure of the parasite may be summarized in the form of a generic description as follows:

PROPARORCHIS Nov. Gen.

Small trematodes with delicate body, widest at center and tapering towards both ends. Oral sucker elongate, protruding; no other sucker present. Esophagus with wide lumen, without pharynx, covered with glandular cells, prominent near posterior end; crura long, sinuous, extending nearly entire length of body. Median glandular diverticulum opposite end of esophagus. Excretory bladder double, short; excretory pore single, subterminal. Genital pore sinistral, ventral, in posterior region. Cirrus sac with slender cirrus and ductus ejaculatorius; seminal vesicle large, pyriform. Testes numerous, irregular, lobed, in intercrural area, between intestinal fork and ovarian complex. Ovary lobed, posterior to testes, chiefly on right side of body; oviduct short, with sperm filled expansion (receptaculum seminis uterinum). Laurer's canal present but no receptaculum seminis. Vitellaria well developed, conspicuous laterally, enveloping intestinal crura on lateral, dorsal and median aspects thruout entire length. Transverse yolk duct with median reservoir between ovary and end of crura. No true uterus present, metraterm extends straight from ootype to pore. Eggs deposited so soon after formation that never more than a single one is seen in the fluke. Egg provided with cap; those in body of worm measure about 80 by 45 μ, in blood vessels of host about 100 by 80 μ. Cleavage well advanced before oviposition; well developed miracidia with conspicuous eye spots in eggs taken from blood vessels of host.

Type and only species: *Proparorchis artericola* from various fresh water turtles.

The data in my possession are not all referable to the single species which has just been described. In details of structure, in regard to the eggs, in the location in the host in which they have been observed, and in some other details, certain specimens differ so distinctly from the account above that I can not at present include them under the same heading. It is possible that they represent different phases in the life cycle of a single species. I am inclined to think the structure of this worm too delicate for one to consider it probable that any part of its life history could be passed in the intestine. But such a transfer must still be kept in mind as a possibility. In my opinion it is much more likely that further study will disclose the presence of several species parasitic in the blood of reptiles and amphibia. I have myself a single specimen of a distome unlike any genus yet described which was found in the course of explorations for the species just described.

RELATED SPECIES OF FLUKES

In a recent paper (G. A. MacCallum, 1919) has given a brief description of an unusual worm found in the *intestine* of a wood turtle at the New York Aquarium. This form is undoubtedly closely related to that described in this paper. To this form MacCallum gave the generic name, *Spirorchis,* but omitted to add any specific designation. In order to insure accuracy of reference, I would suggest that his species be designated *Spirorchis innominata.* MacCallum's description is brief and in some details confused since the dimensions given are clearly wrong and the text does not agree in full with the illustration. On the other hand no one can consider his account without being impressed by the general likeness his species has to that described here. It is important to consider in a comparative fashion the structure of these two forms as a basis for a decision as to the degree of their relationship.

MacCallum's fluke is considerably larger tho of the same general form and apparently also similarly delicate in structure and transparent. The general plan of the organs is much like that in the species just described. The peculiar form and position of the oral sucker in Proparorchis is both described in MacCallum's text and shown in the figure accompanying it; on the other hand, the characteristic angle of the esophagus and crura in this form is not shown or mentioned by MacCallum, and the pharnyx which he describes and pictures near the center of the esophagus is certainly not present at all in the blood fluke I have studied. Many further items in MacCallum's account, like the appearance of the testes and of the seminal vesicle, the size and form of the ovary and of the egg, and the various measurements of the body which do not agree with the description given above, may perhaps be explained as specific differences tho they are not discussed in sufficient detail to make this opinion positive. His very definite statement concerning the location of the genital pore shows a striking difference from the condition in the species described here.

MacCallum states that his parasites were taken from the intestine of a wood turtle *(Chelopus insculptus)* but adds "as will be seen by the color of the contents of its intestines, it is a hematophagic trematode." It is not unlikely that its presence in the intestine was accidental, the result of opening some blood vessel during the dissection, and that in fact that species also is normally an inhabitant of the vascular system. But this is only a tentative opinion.

The converse of that proposition is entirely untenable. One can not maintain the view that the worms I found in the blood vessels are immature forms which might later attain the adult condition in some other location. Several conditions militate against such an explanation. First, large masses of ripe sperm cells are found in the worms, and

occur not only in the seminal vesicle, but also in that portion of the oviduct often termed the receptaculum seminis uterinum. This shows that impregnation has already taken place altho, of course, this might have been self-impregnation which has been observed in encysted trematodes. Secondly, many if not all of the flukes contained in the uterus a single egg which was well started in development. Third, careful microscopic inspection of the ovary and testes gave evidence that these organs in some cases had been functioning for some time. Fourth, the blood vessels contained large numbers of egg shells which enclosed fully developed embryos; these were removed from the vessels and watched in many instances until the miracidia hatched out and swam about in the culture medium. Fifth, all stages in advancing maturity which should be present in adult worms are actually represented in the specimens found, from the young fluke in which the female organs have not yet begun to function actively, to such as show that system at its functional apex whereas the male organs have passed their prime and are already on the decline. Proparorchis becomes fully mature in the blood vessels of the host.

I have endeavored as yet unsuccessfully to get for examination one of the specimens on which MacCallum's description is based. In the light of his description it appears to me necessary to accept his diagnosis as it stands, especially since his previous publications show great care in studying out similar parasites and accuracy in stating the results of such study. Unless the differences are to be explained away as errors in observation, they form an adequate basis for the differentiation of genera even tho these are closely related and should be included in a single subfamily. I have rewritten MacCallum's account in brief taxanomic form in order to facilitate the comparison of that species with the one I have just described.

GENUS SPIRORCHIS G. A. MAC CALLUM

Small species with smooth skin, body widest near center, tapering towards both ends. Anterior sucker small, protruding; no acetabulum present. Esophagus with pharynx near the middle; crura conspicuous because of dark granular contents, extend to near posterior end, sometimes coalescing there. Excretory pore near posterior end. Genital pore median, posterior to tips of intestinal crura. Vitellaria profuse, lateral, along entire length of intestinal crura; transverse yolk duct and median reservoir near posterior limits of yolk glands. Ovary lateral, oviduct long; one large egg measuring 100 by 50 μ,* with thick shell regularly present. Testes irregular, lobed, "in rough spiral column" occupying central region between intestinal crura and followed by large

* MacCallum says 5 to 10μ, an evident error.

conical seminal vesicle which tapers to cirrus (?). Connection of ducts and other organs not seen "on account of black intestines filling the posterior end of the worm."

The genera Proparorchis and Spirorchis are evidently members of a new subfamily to which the name Proparorchiinae may be given. The position of this sub-family deserves further consideration. Its nearest affiliations are to be found on the one hand in a fluke from sea turtles described by Looss and on the other hand in the human blood flukes, the Schistosomatidae.

The first mentioned trematode from the vascular system is *Hapalotrema constrictum* (Leared), most recently studied by Looss (1902:519-). It is a common parasite of *Thalassochelys corticuta,* a sea turtle taken on the Egyptian coast. The eggs are very striking, being large and supplied with long polar processes coiled at the tip. They also occur within the tissues. While these eggs are so conspicuously unlike those of the species described in this paper, yet they enclose an embryo said by Looss to resemble closely that of Schistosoma, as also does the embryo of this species. In general appearance and structure, like Proparorchis, these worms are delicate, thin-skinned and only weakly provided with dermal musculature. In this respect they resemble also the human blood flukes (Schistosomatidae) most strikingly.

In discussing the genus Hapalotrema, Looss (1899:656) comments on its striking similarity to the genus Schistosoma in the alimentary system, the structure of the suckers and the character of the skin. Yet in view of the marked dissimilarity in other respects, he concludes that the likeness is merely convergence due to the place and mode of life since both inhabit the blood stream and feed on the blood. The resemblance to the species reported here is even more striking. The delicate body with scantily developed musculature, the peculiar esophagus, the short female genital canal, the formation and extrusion of eggs one by one, and the well developed ciliated miracidium are all peculiar and characteristic features in which the two forms agree with each other and differ from almost all other known trematodes. While a few of these features are found in the Schistosomes, as noted above and that agreement might be explained as the result of convergence, yet a similar explanation is more difficult to apply to the longer series of structural likenesses between Hapalotrema and Proparorchis.

However, the differences between the two species deserve equal emphasis. First of all, some would list the fact that Hapalotrema is a true distome whereas Proparorchis is a monostome; but to me this is a subsidiary feature since extended studies have led me to the full acceptance of the view presented forcibly by Odhner that the mono-

stomes do not constitute a natural group but represent an assemblage of forms derived from different families of distomes by the reduction and ultimate disappearance of the acetabulum. They are thus alike primarily only in the superficial feature that all possess but a single, anterior sucker. The organ pattern, on the other hand, brings evidence of marked differences in type and indicates clearly relationship to different groups of distomes. Odhner would accordingly classify the monostomes with those distomes to which they are most clearly allied and abolish entirely the major subdivision of Monostomata. For evident practical reasons, such a radical proposal is not likely to be adopted until knowledge of the monostomes has been greatly extended.

The oral sucker of Hapalotrema is described by Looss as flat, saucershaped, projecting above the general surface of the body and scantily developed in musculature. It appears thus as if already in course of elimination, even tho the process is only well begun and the interval that separates the species from Proparorchis is still great.

There are, however, still other and much more significant differences between the two species. In Hapalotrema, according to Looss, the excretory bladder is short and branches just behind the posterior testis.* This does not seem to be the condition in Proparorchis as described above. The genital pore in Hapalotrema lies about in the center of body in the left rather than as in Proparorchis near the posterior end. The eggs are very dissimilar in general appearance, since those of Hapalotrema are provided with two long polar processes on the shell which are spirally twisted at the end. It may be noted that this difference is of the same sort that exists between *Schistosoma haematobium* and *S. japonicum,* tho of course more extremely developed. The egg here has a cover and no such structure is shown for Looss' species.

By far the most striking difference is one that affects the general appearance of the body very radically. At first glance Hapalotrema resembles a common type of distomes; the transverse yolk duct, ovary, seminal vesicle and cirrus lie in the central area and other genitalia are grouped symmetrically about them. In Proparorchis the genital complex lies near the posterior end and the other genitalia lie almost entirely in front of it. A closer analysis shows that Hapalotrema is really of an unusual type morphologically; the two testes are fragmented instead of simple, and one fills the central area anterior to the ovarian complex whereas the other testis occupies the corresponding posterior area.

Conditions in Proparorchis as described above permit of a close comparison with those in Hapalotrema if the posterior region in the latter be reduced by failure of the posterior testis to develop and coin-

* The text says *in front of* the posterior testis, but Looss' figure shows distinctly the other relation.

cident cessation of growth in the entire posterior half of the worm. As the result of this, the ovarian complex lies just in front of the posterior end of the body. The structures in this complex and anterior to it correspond very closely to those in the same regions in Hapalotrema.

Looss (1902) found in the blood vessels of sea turtles four types of eggs easily differentiated from those of Hapalotrema for which no adults were discovered despite the most careful search. These eggs, or at least two of the varieties, are much like those of Proparorchis.

SYSTEMATIC POSITION

In a most interesting and suggestive paper on the Phylogeny of the Bilharzia type, Odhner (1912) established a new family, Harmostomidae, to include the subfamily Harmostominae already worked out by Looss (1899:651-) for a series of genera he had studied, and a second new sub-family Liolopinae in which Odhner includes Liolope Cohn 1902, Helicotrema n.g., and Hapalotrema Looss 1899. The Harmostomidae are placed close to the Schistosomes, which latter in the opinion of Odhner are "with absolute certainty" derived from the former. In my opinion the family Harmostomidae and the sub-family Liolopinae are both unnatural in one and the same particular: the inclusion of the genus Hapalotrema; for this form necessitates a series of exceptions in the descriptions that really do violence to the morphological basis on which those sub-divisions are built. Hapalotrema is unlike all other species in those two groups in the presence of a long esophagus, in the absence of a pharynx, in the form of the ovary and testes, in the absence of a true uterus, and finally in the character of the excretory system which is a most fundamental feature. Hapalotrema must be removed from Odhner's sub-family Liolopinae; it forms with the Proparorchiinae naturally a new family which may be characterized as follows:

FAMILY PROPARORCHIIDAE

Delicate blood inhabiting flukes, with slender, non-muscular body and one or two weak suckers. Testes lobed, multiple, anterior (and sometimes also posterior) to ovarian complex. Laurer's canal present. Ovary lobed; no true uterus; eggs large, thick-shelled, discharged singly.

These forms are certainly related to the human blood flukes, Schistosomatidae, altho not so highly specialized for life in the circulatory system. Finally mention must be made of the peculiar blood-inhabiting fish parasites Aporocotyle and Sanguinicola which have been so thoroly studied and described by Odhner (1911.) These genera show evident morphological likeness to the forms discussed in this paper, and one is fully justified in associating relatively closely the families Aporocotylidae and Proparorchiidae.

Papers Cited

Looss, A. 1895.—Zur Anatomie und Histologie der Bilharzia haematobia (Cobbold). Archiv für mikr. Anat., 46: 1-108.

1899.—Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus Distomum Retzius. Zool. Jahrb., Syst., 12: 521-784.

1902.—Ueber neue und bekannte Trematoden aus Seeschildkröten. Nebst Erörterungen zur Systematik und Nomenclatur. Zool. Jahrb., Syst., 16: 411-894.

MacCallum, G. A. 1919.*—Notes on the Genus Telorchis and other Trematodes. Zoopathologica (N. Y. Zool. Soc.), 1: 81-98.

Odhner, T. 1900.—Aporocotyle simplex n. g. n. sp., ein neuer Typus von ektoparasitischen Trematoden. Centralbl. Bakt. Parasit., Abt. I, 27: 62-66.

1911.—Sanguinicola M. Plehn—ein digenetischer Trematode. Zool. Anz., 38: 33-45.

1912.—Zum natürlichen System der digenen Trematoden. V. Die Phylogenie des Bilharzia-Typus. Zool. Anz., 41 : 54-71.

* Dated 1918 but not distributed until 1919.

Explanation of Plate

c, cirrus; *e*, egg; *gp*, genital pore; *i*, intestine; *lc*, Laurer's canal; *od*, oviduct; *ov*, ovary; *rsu*, receptaculum seminis uterinum; *sv*, seminal vesicle; *t*, testis; *vi*, vitellaria; *yr*, yolk reservoir.

Fig. 1.—*Proparorchis artericola.* Toto mount viewed from ventral surface. Extent of vitellaria shown by dotted line. × 40.

Fig. 2.—Posterior end of same specimen. × 70.

Fig. 3.—Reproductive organs; reconstruction from sections. × 65.

Fig. 4.—Organs near genital pore, showing cirrus partly extended, metraterm, and egg just being formed. × 270.

Fig. 5.—Frontal section of esophagus, bifurcation of crura and diverticulum. × 170.

Fig. 6.—Egg in cleavage from ducts of fluke shown in Figure 1. × 210.

Fig. 7.—Miracidium, viewed in profile, in egg shell from culture; *x*, eye spot in dorsal aspect. × 360.

Fig. 8.—Empty egg shell, showing cap. × 360.

Fig. 9.—Miracidium just out of shell; ciliary coating omitted. × 150.

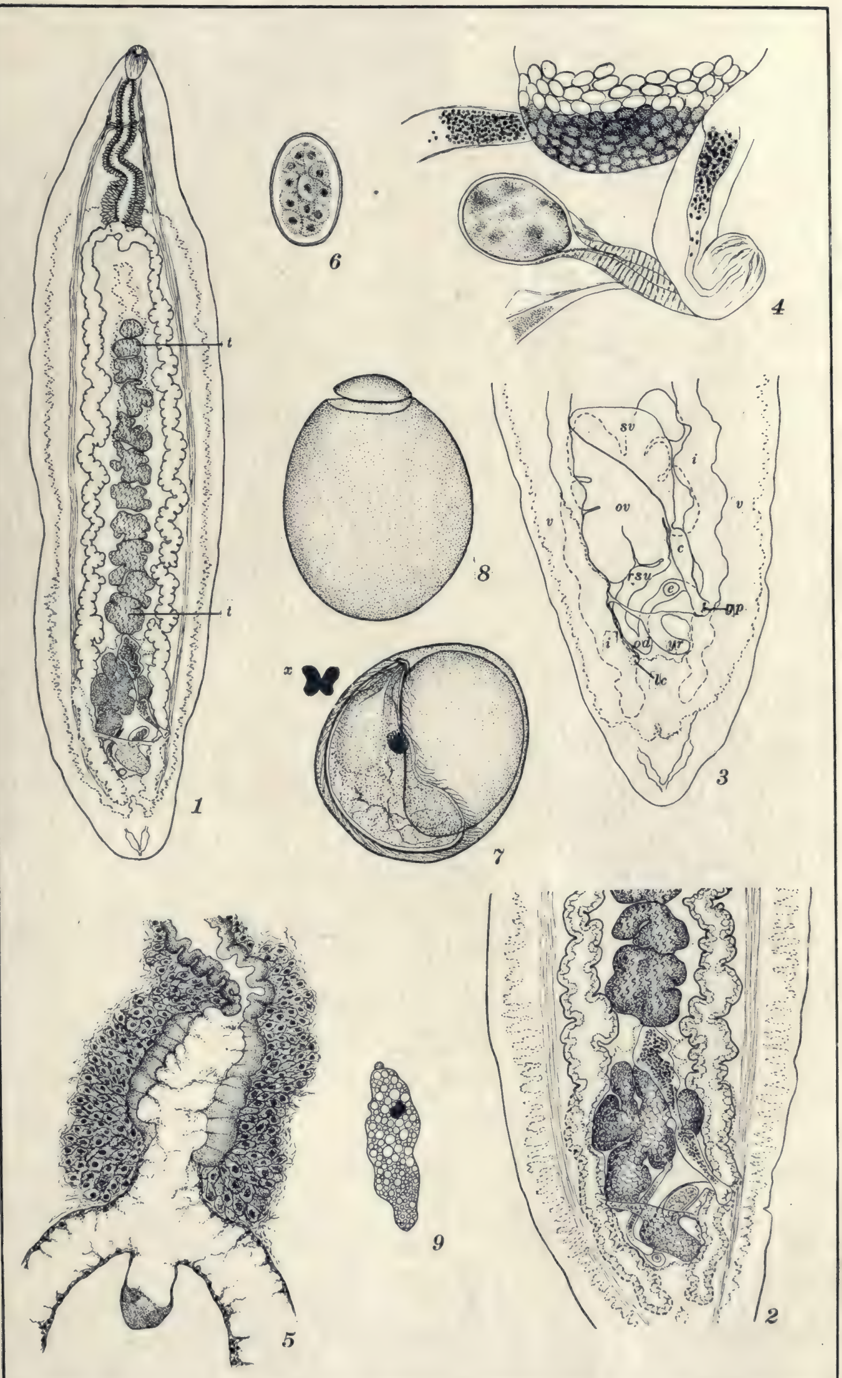


PLATE XII

A NEW AMPHIBIAN CESTODE *

LLOYD B. DICKEY

Few cestodes have ever been described from amphibian hosts. *Nematotaenia dispar* (Goeze 1782) has been reported from Europe by Schmidt (1885), and by Fuhrmann (1895); and from America by Stiles and Hassall (1912; 277). *Taenia pulchella* Leidy 1851 was reported from America in *Bufo americanus,* at the time of its original description. Jewell (1916) described *Cylindrotaenia americana* from America in *Acris gryllis, Rana pipiens, Rana virescens,* and *Bufo lentiginosus.* These three species, together with two Proteocephalids, *Ophiotaenia hylae* from Australia, and *O. schultzei* from Africa (Johnston, 1912), constitute the species so far described from frogs and toads. Johnston (1916: 194) reports the presence of a new species of Nematotaenia in Australia, which has not yet been described. Larval stages of Sparganum have been known to occur in European frogs, but nothing is known of the larval stages of any of the adult cestodes thus far reported in Anurans.

Jewell (1916) has pointed out the discrepancies of Schmidt's description of *Nematotaenia dispar* (Schmidt 1855) as compared with the recognized, original form first described by Goeze (1782), and later by Fuhrmann (1895). It is probable that Schmidt's material was not *Nematotaenia dispar,* since he describes the worm as having a neck, with the greatest diameter at the posterior end, and an oval cirrus sac about twice as long as it is broad. Fuhrmann's description includes a worm devoid of a neck, the greatest diameter of the strobila being at the anterior end, with the cirrus sac about ten times as long as broad.

Lühe (1899: 526) proposed the genus Nematotaenia, to contain *Taenia dispar* Goeze. His first characterization of the new genus, however, appears in a later paper (Lühe 1910), as follows: "Taenien mit unbewaffnetem Scolex, ohne Rostellem, mit drehrundem Körper, der in seinem vorderen Abschnitt etwas dicker ist und nach hinten allmählich dünner und schliesslich fadenförmig wird. Gliederung nur am Hinterende ausgesprochen, wo sich die reifen Proglottiden, die wesentlich länger als breit sind, einzeln ablösen, um dann lebhaft beweglich noch längere Zeit weiterzuleben. Gesclechtsöffnungen randständig, unregelmässig abwechselnd. Hoden in der Zweizahl, dorsal und annähernd symmetrisch. Dotterstock fast genau in der Achse

* Contributions from the Zoological Laboratory of the University of Illinois No. 177.

des Körpers; Keimstock ventral, der Genitalöffnung wenig genähert. Geschlechtswege dorsal von Wassergefässen und Marksträngen. Uterus frühzeitig in einzelne Eikapseln zerfallend, welche je 2-4 (meist 3) Eier enthalten. Eier mit 3 Hüllen. Finnenstadium unbekannt.

"Im Daım von Amphibien. Bisher nur eine Art bekannt."

Jewell (1916: 191) gives the following diagnosis for *Cylindrotaenia*: "Scolex unarmed, without rostellum; reproductive organs single in each proglottid; pores lateral, alternating; vagina and cirrus dorsal to the excretory canals and main nerve trunk; testis one, dorsal; ovary and vitellaria ventral. Uterus breaks up into capsules surrounding the embryos which ultimately pass into two parauterine capsules." *Cylindrotaenia americana* "from the small intestine of various Anura" is designated as the type.

MORPHOLOGY OF THE NEW FORM

The present study is based upon material collected at Oxford, Georgia, in July 1916, from two host specimens of *Bufo lentiginosus*. Of the adult specimens, two bore ripe proglottids, and each measured about 100 mm. in length. Eleven young worms, which varied in length from 3.5 to 5 mm., were collected from a single host.

The worm is cylindrical in form anteriorly. In the region of the strobila where the reproductive organs attain the maximum of development, the segments are oval in cross-section, being compressed laterally (Fig. 1). In a typical specimen, the cross-section is circular in outline at a distance 6 mm. from the tip of the scolex, is oval at a distance of 13 mm., and the circular form is again assumed at a distance of 40 mm., in the region where the eggs have passed into the parauterine capsules and the uterus has broken up.

No external segmentation is apparent in the anterior region of the worm. It occurs rather distinctly from 48 to 60 mm. from the scolex. Here the segments begin to elongate and measure 0.24 mm. in length, and 0.39 mm. in diameter. The last few proglottids of the strobila measure 0.58 to 0.66 mm. in length and 0.18 to 0.24 mm. in diameter.

The scolex is unarmed, spherical, and broader than the neck. In adult specimens it measures 0.52 to 0.62 mm. in diameter. The neck averages 0.48 mm. in width. In young worms the diameter of the scolex is 0.26 to 0.33 mm. The suckers are situated near the tip of the scolex. They are unarmed, and have a diameter in the adult forms of from 0.093 to 0.14 mm. The diameter of the suckers in the young worms is from 0.085 to 0.09 mm. The lumen is directed anteriad and slightly laterad. The scolex is circular in cross-section, except through the region of the suckers, where it is slightly oval.

Two shallow grooves, extending from the tip of the scolex to the base of the suckers, occur on opposite sides of the scolex.

From the material at hand it appears that the cuticula is composed of two layers of equal thickness. The outer layer stains more heavily. The cuticula is 4 to 5μ in thickness and is supported by a delicate basement membrane. The subcuticula consists of cells 32μ long and 4μ in diameter. The nuclei are large and stain less heavily than the rest of the cells.

The well developed longitudinal muscles are arranged in a single layer. They separate the parenchyma into a cortical and a medullary area. The latter averages about 0.296 mm. in dorso-ventral diameter and about 0.222 mm. in lateral diameter in the region where the reproductive organs attain their maximum development. The longitudinal strands occur at approximately 0.083 mm. from the cuticula. About five or six small strands go to make up a large bundle. Between fifty and sixty of these bundles occur at more or less regular intervals. Many of them extend from one segment to another. Between subcuticula and cuticula the longitudinal fibers of the subcuticular muscles can barely be discerned. No trace of dorso-ventral muscles was found. The muscle strands of the longitudinal system are large and numerous, and are massed together at the tip of the scolex. There are also transverse muscles running concentric to the basement membrane of the suckers.

The ventral excretory canals are about 32μ in diameter. They pass lateral to the ovary about 50μ from the nearest longitudinal muscle strands. Commissures of the ventral excretory canals may be seen in the region where the reproductive organs first differentiate from the medullary parenchyma. These have a diameter of about 3μ. The dorsal excretory canals are very small, and are seldom discernible. They appear most prominently in the region where the testes first become differentiated and here they often have a diameter of 8μ. In the region of the scolex, they sometimes anastomose with the other excretory canals. The excretory system is continuous from one proglottid to another throughout the strobila. In the ripe proglottids the ventral canals present undulations, due to their position exterior to the developing parauterine capsules.

The two main lateral nerve trunks run parallel to and midway between the dorsal and ventral excretory vessels.

The genital rudiments are first seen at about 2 to 3 mm. from the tip of the scolex. They appear here merely as a dark streak running through the center of the proglottids. The testes are the first organs to become differentiated from the parenchyma. The ovary arises next, and the vitelline gland forms dorsad of the latter immediately afterwards. The testes are distinguishable 3 to 4 mm. from the sco-

lex. No external segmentation is apparent at this point. The internal or genital segmentation can be distinguished, however, the segments being 0.04 to 0.05 mm. long. All of the reproductive system, except the cirrus sac and vagina, is accommodated within the medullary parenchyma (Fig. 1). The genital pores are lateral and marginal, and alternate irregularly. The cirrus sac and vagina open into the genital atrium dorsal to the excretory canals and main nerve trunk.

The male organs are situated dorsally in the proglottid. The testes, two in number, are about 67μ in diameter at their greatest development. They are lenticular in shape and circular in cross-section, the antero-posterior thickness averaging 40μ. This compression in the antero-posterior direction may be due to the contraction of the worm when killed, and the organs are probably spherical in shape in the live worm. A thin membrane surrounds each testis and is continuous with the walls of the vas deferens. The vasa efferentia, one from each testis, meet to form the vas deferens, near the testis on the poral side of the proglottid. After forming one or two short loops during its course, it passes into the cirrus sac (Fig. 1). The cirrus proper is surrounded by parenchymatous tissue composed of small cells with spherical nuclei. The cirrus pouch is flask shaped and is about one and a half times as long as it is broad. The length averages 48μ, and the diameter 31μ.

The ovary is a spherical organ, lying in the ventral half of the medullary region and slightly to the poral side of the proglottid. The diameter averages 67μ. Numerous spherical cells, each enclosed in a capsule, make up the organ. The capsules average 12μ in diameter. The vitelline gland, also spherical in shape, lies dorso-lateral to the ovary but ventral to the genital pore. Its diameter averages 35μ. The vitelline duct is directed laterad and meets the oviduct, which extends laterally and dorsally. The oviduct is continuous with the vagina, which in turn leads ventral to the cirrus pouch, running adjacent to it from the inner end of the latter.

The beginning of the uterus can be distinguished in proglottids 12 to 14 mm. from the scolex. The uterus is horseshoe shaped in appearance (Fig. 2). It arises from the medullary parenchyma and soon almost completely surrounds the vitelline gland. The ovary and testes break down at the same time, the ovary disappearing before the testes. At the maximum development of the uterus, about 18 to 20 mm. from the tips of the scolex, the eggs are 16μ in diameter. They are in the early stages of cleavage. The internal segments at this place are about 0.11 mm. long. The uterus breaks down early, about 22 to 24 mm. behind the scolex, and is replaced by the parauterine organs.

The parauterine organs arise from the parenchyma adjacent to the uterus. The strands of the meshwork of which they are com-

posed soon arrange themselves parallel to the uterus on the anterior side of the proglottid. The whole structure from this stage on grows very rapidly. The tissue migrates inwards, replacing the uterus by capsules, and surrounding the egg at the same time (Figs. 5, 6). The capsules are early seen to have well defined walls. All trace of the ovary disappears, but the remnants of the testes apparently persist as long as any trace of the uterus itself can be found.

From eight to twelve truncated or flask-shaped cones appear, arranged in two parallel rows (Figs. 3, 4), one row dorsal, the other ventral. There are from four to six capsules in each row, their usual number being five. A sort of raphe, which is composed of numerous, small, spherical and thickly massed cells, staining a dark gray with hematoxylin, separates the capsules of the two rows as they come together in the center of the proglottid. The basal portion of the capsules is in the posterior portion of the proglottid and the longitudinal axes of the cones correspond to the longitudinal axis of the worm. A meshwork of fine fibers together with a fine granular tissue adjoins the capsules in the basal portion. The apex, capped in each instance by the darkly-stained cell-gland secretion, is situated in the anterior portion of the proglottid. The minute cells, from which the secretion is emitted, are easily distinguished at the most anterior end of the apparatus. The length of the cones increases as the proglottids become elongated. At the time the cones attain their greatest length, a distance of 60 to 70 mm. from the head, they measure approximately 0.11 mm. in length. The width of the base of a cone averages 0.06 mm. in lateral diameter, and 0.09 mm. in dorso-ventral diameter. The capped secretion averages 0.3 mm. in width and 0.07 mm. dorso-ventrally.

The eggs, occupying the basal portion of a cone, are three to six, usually four, in each capsule. They are oval, averaging 43μ in length, and 31μ in diameter. The embryos are still in the spherical stage, and average 19μ in diameter. The eggs have a shell about 3μ in thickness. Only a single thin membrane could be distinguished surrounding the embryo.

As the proglottid elongates the cones change in position and shape (Fig. 7). By the time the proglottids have become detached, the cones have become more spherical, especially in the apical portions, which now appear larger than the basal regions. This growth takes place at the expense of the surrounding parenchyma, the latter furnishing the nourishment necessary for the growth of the capsule. The fact that the parenchyma is much less dense at this stage than in earlier stages is easily seen in cross-sections of the proglottids. Some of the eggs migrate from the base of the cone into the apex. The bases of the cones remain clustered together, while the apical

portions spread apart from each other irregularly in all directions. This is caused by the total disappearance of the raphe composed of the numerous small, spherical cells, which heretofore held the capsules together in the two parallel rows. The capped secretion spreads out over the apex of each capsule. Portions of the fibrous and granular tissue at the bases of the cones may become broken off and migrate into the parenchyma. This arrangement may be distinctly seen in detached proglottids.

Since the living parasites were not obtained nothing is known of their further development.

COMPARISON OF FORMS

The form under consideration bears striking resemblances to *Nematotaenia dispar* (Goeze 1782). It is similar in external form and in the limitation of marked segmentation to the posterior end. The reproductive organs are similar in shape and position. There are two testes, and the ovary, vitellaria, and uterus are single in both cases. A difference occurs in the shape of the cirrus pouch. In *Nematotaenia* this is tubular, and about ten times as long as broad, while in the present form the cirrus pouch is flask-shaped, and about one and a half times as long as it is broad. The uterus in both forms is horseshoe-shaped, and breaks down early. The most marked differences between the two forms lie in the development, position, and number of the parauterine organs. In Nematotaenia there are developed a varying number of small parauterine organs. Ripe proglottids show from thirteen to thirty capsules, scattered irregularly throughout the parenchyma. In the new form, the number of parauterine organs is limited, the mature proglottids showing eight to twelve capsules, arranged in the two parallel rows.

The only description extant of *Taenia pulchella* Leidy 1851, is much too meager to permit of a satisfactory comparison with other forms. It has general similarities with the form under consideration, such as its occurrence in the same host genus and its external appearance.

Certain marked similarities occur between the new species and *Cylindrotaenia americana* Jewell 1916. In Cylindrotaenia the cylindrical form also occurs, and the segmentation of the strobila is evident at the posterior end only. The musculature of the two species is very similar. While in both forms the male reproductive organs are limited and definite in number, in Cylindrotaenia there is only one, and in the new form two, testes. In the former the vas deferens leads straight to the cirrus, while in the new worm there are various undulations. The female reproductive organs are very similar.

Again, the most marked difference between the two species occurs in the number and position of the parauterine capsules. In Cylindrotaenia two truncated cones appear, one dorsal and one ventral, the parauterine capsules being thus definitely limited. In the form here described, the capsules are also limited, although not as definitely, and they are more numerous than in Cylindrotaenia, being eight to twelve in number, with the regular arrangement previously noted.

SYSTEMATIC POSITION

In the Revision of the Cyclophyllidea (Lühe 1910) a new family, Nematotaeniidae, has been created for the reception of *Nematotaenia dispar*. This classification does not take into consideration certain essential characters of development and morphology, which relate it more closely to other forms.

Fuhrmann (1908:29) and Ransom (1909:88) have placed Nematotaenia in the subfamily Paruterininae, with six other genera: Paruterina Fuhrmann, 1916; Culcitella Fuhrmann, 1916; Rhabdometra Kholodkovski, 1900; and Biuterina Fuhrmann, 1902. Following is Ransom's diagnosis of the subfamily (Ransom, 1909:85): "Hymenolepididae; scolex usually armed, rarely without rostellum. A single set of reproductive organs in each segment. Uterus simple or double with a single parauterine organ or multiple with several parauterine organs, into which the eggs pass in the final stage of development of the segment. Adults in birds and amphibia." Paruterina Fuhrmann, 1906, is designated as the type genus.

Nematotaenia differs greatly from the other six genera included in this subfamily. This is evidenced by its cylindrical form, its two testes, as compared with the numerous and indefinite number in the other genera, the early degeneration of the uterus and its numerous parauterine capsules.

Jewell (1916), after describing Cylindrotaenia, creates a new subfamily for its reception, and includes Nematotaenia. This new subfamily is characterized as follows: "Cylindrotaenianae: Cylindrical Dilepinidae having one or two dorsally placed testes, ovary and vitellaria ventral, vitellaria dorsal to ovary. Proglottids distinct at the posterior end only. The uterus breaks down early and the embryos are later enclosed in parauterine capsules." The same writer considers *Taenia pulchella* Leidy as probably belonging to this subfamily.

It is evident from the previous description of the new form, that it belongs in this subfamily. However, because of the great differences in the number and arrangement of the parauterine capsules, it cannot be placed in either the genus Nematotaenia or the genus Cylindrotaenia. The definite, regular arrangement of the capsules in two parallel

rows would make it generically distinct. It is therefore necessary to create a new genus for the reception of this form, the diagnosis of which would read as follows: Distoichometra: Scolex unarmed, without rostellum. Body generally circular in cross-section, or nearly so. Genital pores alternating irregularly. Testes two in number, dorsal. Cirrus pouch approximately one and one-half times as long as broad. Ovary ventral. Uterus horseshoe-shaped, breaking up early into two parallel rows of egg-capsules, 4 to 6 in a row. Capsules hold 3 to 6 eggs each. After breaking up of uterus, capsules remain clustered together and do not become scattered throughout the parenchyma.

Type species *Distoichometra bufonis,* gen. nov., sp. nov., with characters of the genus; adult from the intestine of *Bufo lentiginosus.*

The writer wishes to express his thanks to Professor Henry B. Ward, under whose supervision the work was done, for his helpful criticisms and suggestions.

Literature Cited

Fuhrmann, Otto. 1895.—Die Taenien der Amphibien. Zool. Jahrb., Anat., 9:207-216.

1908. Die Cestoden der Vögel. Zool. Jahrb., Suppl. 10, 232 pp.

Goeze, J. A. E. 1782.—Versuch einer Naturgeschichte der Eingeweidewüermerthierischer Körper, Blankenburg. 471 pp., 35 pls.

Jewell, Minna E. 1916.—Cylindrotaenia americana nov. sp. from the Cricket Frog. Jour. Paras., 2:181-292.

Johnston, T. Harvey. 1912.—Notes on Some Entozoa. Proc. Roy. Soc. Queensland, 24:63-91.

1916.—Helminthological Notes. Mem. Queens. Mus., 5:186-196.

Leidy, Joseph. 1851.—Contributions to Helminthology. Proc. Acad. Nat. Sci. Phila., 5:239-244.

Lühe, M. 1899.—Zur Kentniss einiger Distomen. Zool. Anz., 22:524-539.

1910.—Parasitische Plattwürmer. II: Cestodes. In Die Süsswasserfauna Deutschlands, 18; 153 pp., 174 figs.

Ransom, B. H. 1909.—The Taenoid Cestodes of North American Birds. Bull. U. S. Nat. Mus., no. 69.

Schmidt, Otto. 1855.—Ueber den Bandwurm der Frösche *Taenia dispar* und die geschlechtslose Fortpflanzung seiner Proglottiden. Zeitsch. ges. Naturw., 5:1-13.

Stiles, C. W., and Hassall, A. 1912.—Index Catalogue of Medical and Veterinary Zoology; subjects Cestoda and Cestodaria. Bull. 85, U. S. Public Health and Mar. Hosp. Serv.

Weinland, D. F. 1858.—Human Cestoides. An Essay on the Tapeworms of Man. Cambridge, 93 pp., 12 figs.

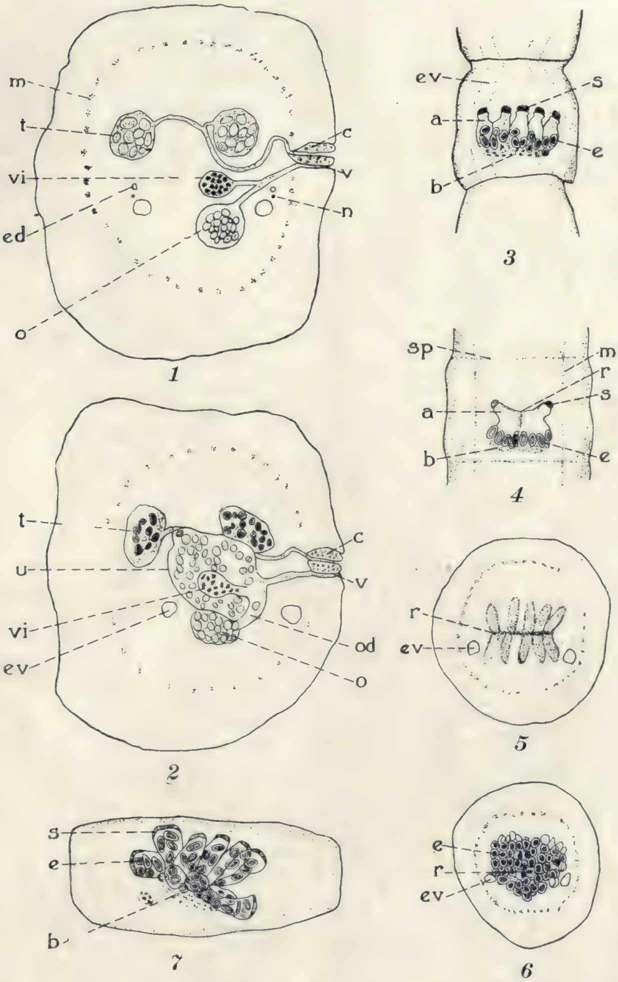


PLATE XIII

EXPLANATION OF PLATE

a, Apical and *b,* Basal portion of parauterine organ; *c,* Cirrus pouch; *e,* Eggs in parauterine organ; *ed,* Dorsal excretory canal; *ev,* Ventral excretory canal; *m,* Longitudinal muscle; *n,* Longitudinal nerve; *o,* Ovary; *od,* Oviduct; *r,* Raphe; *s,* Gland-cell secretion; *sp,* Septum between proglottides; *t,* Testis; *u,* Uterus; *v,* vagina; *vi,* Vitelline gland.

Fig. 1.—Cross-section of mature proglottid, $\times$ 145.

Fig. 2.—Later stage than fig. 1, $\times$ 145.

Fig. 3.—Dorsal view of ripening proglottid with parauterine capsules. Toto mount.

Fig. 4.—Lateral view of slightly earlier stage than figure 3. Toto mount.

Fig. 5.—Cross-section through apical portion of parauterine organs. $\times$ 65.

Fig. 6.—Cross-section through basal portion of parauterine organs. $\times$ 65.

Fig. 7.—Ripe, detached proglottid. Toto mount.

MICROSPORIDIA PARASITIC IN COPEPODS *

R. Kudo

While working on Cnidosporidia of various aquatic animals at Spring Valley, New York, during the summer of 1920, my attention was called to microsporidian infections in *Cyclops albidus* and *Cyclops fuscus.* Notwithstanding the fact that an enormous number of papers dealing with the occurrence, taxonomy and biology of several species of copepods have been published, it is strange to find but a few brief notes recording the occurrence of microsporidia-like parasites in the animals under consideration. Aside from one brief paper by Schröder (1914), all the others, which will be reviewed later, were published in Europe between 1887 and 1895 and the microsporidian nature of the forms described, is open to question. I have failed to find any record from any other land than Europe. In view of the circumstances, it seems worth while to describe the microsporidia which have come under my observation, and to review the old European records.

Although six species, i. e., *Cyclops albidus, C. fuscus, C. ater, C. phaleratus, C. prasinus* and *C. serrulatus,* were examined, only the first two species were found to be infected by the microsporidian parasites. In each collection, the number of individuals of *Cyclops albidus* predominated over the other five species. The absence of the infected animals in the latter four species may be due to the small number of individuals examined, and not to the specific difference in the host. It seemed to me that there could not be any special factor or factors that might have caused the infection to occur among the first two host species.

Before the examination for the microsporidian parasites, each host animal was placed on a slide, and identified by means of Marsh's key (1918). The animal was then slightly pressed under a cover glass, and examined microscopically in either fresh and stained smears or section preparations. The methods of fixation and staining, and also of the extrusion of the polar filament were exactly same to those which I have used in my previous studies (Kudo, 1920, 1920a).

Nosema cyclopis nov. spec.

Habitat: In the fat bodies and reproductive organs (?) of *Cyclops fuscus.* Spring Valley, New York (August).

Two, one male and the other female, out of twenty-two individuals collected from a creek on August 20, were found to harbor the Micro-

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* Contributions from the Zoological Laboratory of the University of Illinois, No. 178.

sporidian. Six fixed specimens from Conger's Lake and twenty-four from the outlet of the latter were free from the infection.

The two infected animals were strikingly whitish opaque in color compared with the normal ones. The host did not suffer any decrease of the activity. This is probably due to the fact that the parasites do not attack the muscle cells. Concerning the effect of the parasitism upon the host body, I cannot make any definite statement as the number of the infected animals were small and they were not kept alive for any length of time under observation. However, there seems to be little doubt as to the fatal outcome of the infection.

The spores are pyriform (Figs. 1 to 7). The anterior end is rounded at its tip, while the posterior margin is broadly rounded. The spore membrane is very thin. The spore is less refractive than that of any species which I have studied up to the present. In cross-section, the spore is circular (Fig. 4). The broadest portion of the spore is located near the posterior extremity. The shape and size are

Figs. 1 to 7. Spores of *Nosema cyclopis* nov. spec. 1 to 3, fresh spores. 4, an optical cross-section. 5, a spore stained with Giemsa's stain. 6, a spore stained with Heidenhain's iron hematoxylin. 7, a spore mechanically compressed and stained with Giemsa's stain, the greater part of the polar filament is not shown. × about 2350.

constant. In the fresh state, there is always seen an oval clear space at the posterior end of the spore (Figs. 1 to 3). The rest of the cavity of the spore is filled with finely granulated cytoplasm. When stained (Figs. 5 and 6), there appear two deeply stained masses in the spore, a sporoplasm which looked as a clear space in the fresh spore and the coiled polar filament as was the case in *Thelohania magna* (Kudo, 1920) or *Nosema apis* (Kudo, 1920a). The polar capsule could be detected in some spores where the polar filament was extruded (Fig. 7). The polar filament seemed to be coiled up in a way similar to *Thelohania magna.* The doubly coiled condition which was recognized in *Nosema apis,* was not observed. Fresh spores measure 4.2 to 4.7μ long by 2.7 to 3μ. The length of polar filaments extruded under the influence of mechanical pressure, fixed with sublimate alcohol and stained with Giemsa's stain, varies from 75 to 100μ.

Nosema infirmum nov. spec.

Habitat: In the fat body, reproductive organs and muscle of *Cyclops albidus.* New City pond and the outlet of Conger's Lake (August and September).

This species was noticed in one out of twelve host specimens obtained from New City pond on August 29, and in twenty-one out of 153 collected in the outlet of Conger's Lake on September 6. The animals collected in the same creek from which *Cyclops fuscus* infected by *Nosema cyclopis* were obtained, proved to be free from the infection. The infected animals were as strikingly whitish opaque in color as in the case already mentioned. However, they showed a marked decrease in activity compared with normal ones. While the apparently uninfected individuals were hard to capture with a pipette, the infected animals were easily caught by the same means.

The effect of the Microsporidian upon the host body in this case seems to be fatal. In the collection from the latter locality, I found fifteen dead animals completely filled with the spores and extremely whitish opaque in appearance, which condition made such animals conspicuously visible against the brownish bottom soil of the aquarium

Figs. 8 to 17. Spores of *Nosema infirmum* nov. spec. 8 to 11, fresh spores. 12, an optical cross-section of a fresh spore. 13 and 14, young spores, stained with Giemsa's stain. 15, spore stained with Heidenhain's iron hematoxylin. 16, a spore stained with Giemsa's stain. 17, a spore mechanically compressed and stained with Giemsa's stain, the greater part of the polar filament is not shown. × about 2350.

in which they were kept. As no observations were carried out, due to the lack of time at that time, on the infected animals kept in the aquarium for any length of time, it is hard to determine the cause of the death of these *Cyclops albidus*. I am, however, led to consider that the Microsporidian infection was more or less directly responsible for the death of the host animals.

The spores are pyriform (Figs. 8 to 17). The anterior end is rounded at the tip. The posterior extremity is either more pointed or rounded than the anterior. The shape of the spore is however distinctly different from that of the species just described. The spore membrane is thin, but is slightly thicker than that of *Nosema cyclopis*. In cross-section, the spore is circular (Fig. 12). The broadest part

passes through the middle of the long axis of the spore. The size and shape vary to a certain extent. In the fresh state, there is seen a clear space which is either oval or irregularly triangular in form, at or near the posterior extremity of the spore (Figs. 8 to 11). The rest of the contents of the spore appear finely granulated. When stained, the cytoplasm is seen to be located at the posterior end where a clear space was noticed in the fresh state. The polar filament is more distinctly visible even in unextruded condition than the former species (Figs. 15 and 16). The fresh spores measure 5.6 to 6.4μ long by 3μ broad. The length of the polar filament, as determined in the same way mentioned in the first species, varies from 90 to 115μ.

Observations upon the schizogony and sporogony are insufficient and I cannot give satisfactory full accounts of the changes that take place. As far as the observation up to the present date, is concerned, the vegetative forms of the two species, the schizonts as well as sporonts, can not be distinguished from each other unless spores are found with them, in which case, the distinction between the two species is comparatively easily done.

The youngest schizonts are rounded bodies, each with a single deeply stained chromatic mass. The body increases in size as the nucleus multiplies repeatedly, forming spherical, oblong or elongated bodies with 2, 3, 4, 5 or 6 nuclei. The ultimate products seem to be uninucleate rounded sporonts. Each sporont develops into a single spore which characterizes the genus Nosema. The development seems to be much different from *Thelohania magna* or *Thelohania illinoisensis,* but similar to that of *Nosema bombycis* (Kudo, 1916) or *Nosema baetis* (see my account).

The difference in the form, appearance and size of the spores of the two species, leads me to record them as two distinctly different species.

An examination of previous records shows that in every case the absolute proof of the microsporidian nature of the parasites, which is, above all, the presence of a polar filament in each spore, is lacking. Strictly speaking, therefore, one can not say whether any Microsporidia were previously found in copepods or not, without reexamining the preparations if such can be obtained.

The nature of "Pilzsporen" found and mentioned by Claus (1863: 87) in the body cavity of *Cyclops* species, remains undetermined, although he seemed to have seen bodies similar to the spores of *Nosema bombycis.*

Moniez (1887) described briefly the following three species, two of which were later studied by Pfeiffer (1895) and the other by Schröder (1914).

Nosema (?) *parva* Moniez

1887, *Nosema parva,* Moniez, 1887: 1313.

1895, *Glugea leydigii,* Pfeiffer, 1895: 83, 86.

Habitat: *Cyclops* spp. at Lille and Weimar.

Pfeiffer states that the species invades the fat bodies and reproductive organs.

Vegetative form: Moniez simply states that the sporogenic masses are relatively voluminous. Pfeiffer describes "cysts" with spores are rounded or elongated. The "cyst" with macrospores is about half the size of that with microspores.

Spore: After Moniez: oval, with a clear space regularly at one end. Size 3.5μ by 2μ. After Pfeiffer: pyriform, with a clear spot at rounded end. Size 8μ by 5μ.

Remarks: The identity of the two forms is very doubtful because of the unusual difference in size of spores. The description is so brief and incomplete that one cannot place the species definitely to any genus. The original nomenclature is retained.

Thelohania acuta (Moniez)

1887, *Microsporidium acuta,* Moniez, 1887: 1314.

1914, *Thelohania acuta,* Schröder, 1914: 324-327.

Habitat: *Cyclops gigas* and *Daphnia pulex* at Lille and in Germany.

Schröder states that the infection was chiefly noticed in the fat bodies.

Vegetative form: Not described.

Spore: After Moniez: The spore terminates in a sharp point, and measures 5μ long by 2μ broad. After Schröder (on fixed specimens): Elongated pyriform; one end terminates in a blunt point, while the other is rounded. Circular in cross-section. A pyriform polar capsule not longer than the half of the spore, is seen at the anterior half. The rest of the spore is filled with cytoplasm which contains a spherical vacuole at the posterior end. The polar filament could not be extruded. Size same to that measured by Moniez.

Thelohania virgula (Moniez)

1887, *Nosema virgula,* Moniez, 1887: 1313.

1895, *Glugea virgula,* Pfeiffer, 1895: 86.

Habitat: *Cyclops* spp. at Lille and Weimar.

Vegetative form: Moniez states simply that the sporogenic masses reach 30μ by 20μ.

Spore: After Moniez: pyriform, one end rounded, the other sharply pointed. Anterior part is often bent to one side. A large vacuole at the rounded end. Size 8μ by 3μ. After Pfeiffer: spores (eight in his figure) are always arranged in a stellate form. Size 8μ by 5μ.

Remarks: The identity of these two forms is again doubtful. Pfeiffer's figure suggests that the species, if it is a Microsporidian, may belong to the genus Thelohania. It is provisionally placed there.

A parasite seems to have been observed by Schmeil (1891), Schewiakoff (1893) and Pfeiffer (1895).

Glugea schmeili Pfeiffer

1891, Myxosporidia, Schmeil, 1891: 19-21.

1893, Myxosporidia, Schewiakoff, 1893: 15-25.

1895, *Glugea schmeilii,* Pfeiffer, 1895: 84-86.

Habitat: *Cyclops* sp., *Diaptomus coeruleûs* and *D. salinus* at Halle and Weimar.

Schewiakoff states that the species forms conspicuous masses in the body cavity and the spores are found "on" the muscle. Both Schmeil and Schewiakoff remarked on the opacity of the body of the infected animals.

Vegetative form: Schewiakoff studied the continuous changes in living animals using apochromatic objective 4 mm., and states as follows: The amoeboid forms are found in the body cavity of the host. They are uninucleate. The cytoplasm is finely granulated, and forms hyaline lobose pseudopodia from any part of the surface. A nucleus and a contractile vacuole are present. Size varies from 7 to 20μ long by 3 to 6μ broad. They creep around on the epithelial and muscle cells. They become encysted. Plasmotomy of two or three individuals was frequently noticed. In the cyst, the nucleus or nuclei divides into a large number. Each daughter nucleus becomes the center of a spore.

Spore: After Schewiakoff: oval, measuring 3.2 to 4μ in length. At the broader end, a homogeneous, spherical (1.6μ in diameter) and refractive nucleus is located. Each "spore" further divides into two, the nucleus undergoing mitosis (number of chromosomes eight).

Remarks: It is highly doubtful if the amoebula with a contractile vacuole, mentioned by Schewiakoff, is a stage in the life cycle of the present paratite. Assuming that this is a Microsporidian, then it should be placed in the genus Glugea, as Pfeiffer did.

The following two more species described by Fritsch (1895) who thought them to be Microsporidia, and placed them in the genus Glugea, are much more doubtful forms than the preceding species.

Glugea colorata Fritsch

1895, *Glugea colorata,* Fritsch, 1895: 80-81.

Habitat: *Diaptomus gracilis* in Austria. The parasite formed masses of olive green or burnt sienna in color.

"Cyst" contained only 5 or 6 spores.

Glugea rosea Fritsch

1895, *Glugea rosea,* Fritsch, 1895: 81.

Habitat: *Cyclops strenuus* in Austria (June). One host animal rose-colored was observed.

Spore: Two kinds: one, smaller, oval with a somewhat pointed end and yellowish in color; the other, larger and elongated pyriform with or without vacuole.

Compared with these six ambiguous species of Microsporidia (?), the two American forms described differ greatly. Hence I have designated them by new specific names. On obtaining more material, I shall try to work out the life history of the Microsporidia.

SUMMARY

1. Two new Microsporidia, *Nosema cyclopis* nov. spec., parasitic in *Cyclops fuscus,* and *Nosema infirmum* nov., parasitic in *Cyclops albidus,* are described.

2. The effect of the infection of *Nosema infirmum* upon its host body, seems to be fatal.

3. The former papers which recorded the occurrence of Microsporodia-like parasites in copepods, are reviewed.

Papers Cited

Claus, C. 1863.—Die frei lebenden Copepoden. Leipzig. 230 pp., 37 pl.

Fritsch, A. 1895.—Ueber Parasiten bei Crustaceen und Räderthieren der süssen Gewässer. Bull. int. acad. sci. Emp. Fr. Joseph I, 2: 79-85, 9 fig.

Kudo, R. 1920.—On the structure of some Microsporidian spores. Jour. Parasit., 6: 178-182; 11 fig.

1920 a.—Notes on Nosema apis Zander. Jour. Parasit., 7: 85-90; 14 fig.

Moniez, R. 1887.—Observations pour la revision des Microsporidies. C. R. acad. sci., 104: 1312-1314.

Pfeiffer, L. 1895.—Nachträge zu: Die Protozoen als Krankheitserreger. Corresp.-Blätt. allg. ärztl. Verein Thüringen, 24: 1-32, 48-66, 74-93, 117-122.

Schewiakoff, W. 1893.—Ueber einige ekto- und entoparasitische Protozoën der Cyclopiden. Bull. Soc. Imper. Natur. Moscou, 7: 1-29; 1 pl.

Schmeil, O. 1891.—Beiträge zur Kenntnis der Süsswasser-Copepoden Deutschlands mit besonderer Berücksichtigung der Cyclopiden. Leipzig. Inaug. Dissert., 40 pp.

Schröder, O. 1914.—Beiträge zur Kenntnis einiger Microsporidien. Zool. Anz., 43: 320-327; 7 fig.

EFFECTS OF SECRETIONS OF CERTAIN PARASITIC NEMATODES ON COAGULATION OF BLOOD.[1]

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INTRODUCTION

The fact that certain parasitic nematodes, especially some members of the family Strongylidae, a group that includes the hookworms, have the power of lacerating the intestinal mucosa, places these parasites in the category of serious pathogenic agents. In considering the question of their pathogenicity, in addition to the damage done by the abstraction of blood and by the mechanical injury to the mucosa of the intestine, caused by their bites, including the entrance of bacteria, it is important to consider the possible effects of the secretions of the worms on the intact as well as on the injured intestinal mucosa and the general effects on the host of the absorption of these secretions into the circulation. Among the toxic products elaborated by these nematodes substances that retard coagulation of blood have been found in certain species. That these substances are responsible for the persistent oozing of blood from wounds inflicted by hookworms and related nematodes, appears probable.

A record and discussion of the writer's experiments on effects of extracts of certain nematodes on coagulation of blood is given in the following pages. No attempt has been made to correlate the results of these experiments with theories of blood coagulation.

REVIEW OF LITERATURE

That the secretions of certain nematodes have the power of retarding coagulation of blood *in vitro* was first shown by Loeb and Smith in 1904. These investigators found that extracts of *Ancylostoma caninum* in physiological salt solution inhibit the coagulation of dogs' blood *in vitro* for periods which vary with different samples of blood, the maximum period of delay in coagulation observed by these writers being about twenty-four hours. Loeb and Smith found, moreover, that the substance involved in this process is present in the anterior half of the worms and is completely absent in the posterior half. The substance was found by these writers to be highly resistant to heat since

1. This paper was read before the Helminthological Society of Washington, on November 20, 1920, at the School of Hygiene and Public Health of Johns Hopkins University, Baltimore, Md.

2. Resigned, December 15, 1920.

fifteen minutes' boiling merely weakened but did not destroy its power to inhibit coagulation of blood.

Although the work and conclusions of Loeb and Smith are questioned by Liefmann (1905), experimental work by Loeb (1906) and Loeb and Fleisher (1910) showed quite conclusively the presence in the anterior portion of the body of *Ancylostoma caninum* of a substance that retards coagulation of dogs' blood, and confirmed the conclusions of the earlier work of Loeb and Smith.

Weinberg (1907) in the course of his investigations on effects of extracts of horse strongyles, belonging to the genus Strongylus, on the blood of the horse, found that physiological salt solution extracts of triturated specimens of these worms inhibited coagulation of horses' blood, since mixtures of the freshly drawn blood and extracts in question were still uncoagulated after four days.

Aside from the experimental work with extracts of worms belonging to the genera Ancylostoma and Strongylus, there appears to exist some evidence in favor of the view that the fluid which occurs in the body cavity of worms belonging to the genus Ascaris has the power of inhibiting to a certain extent coagulation of blood *in vitro*. Leroy (1910), experimenting with dogs, and Weil and Boyé (1910), experimenting with rabbits, found that the blood of animals which had been injected with the body fluid of ascarids coagulated more slowly than the blood of non-injected animals, but Weil and Boyé failed to observe that the fluid had any effect on the coagulation of rabbits' blood *in vitro*. Flury (1912), on the other hand, found that the fluid delayed coagulation of dogs' blood and human blood *in vitro*.

EXPERIMENTS BY THE WRITER

Experiments by the writer have been made with physiological salt solution extracts of *Strongylus vulgaris, Strongylus edentatus, Bustomum phlebotomum, Bustomum trigonocephalum, Stephanurus dentatus, Oesophagostomum columbianum, Dictyocaulus filaria, Haemonchus contortus, Ascaris lumbricoides, Ascaris equorum,* and *Belascaris* sp. The extracts in question were prepared from specimens collected shortly after the death of the host. The specimens were washed in physiological salt solution, dried between layers of filter paper and exposed to room temperature or to a temperature of 37° C. until they became sufficiently crisp to be pulverized. A quantity of powder was then added to physiological salt solution in a test tube, the contents were thoroughly shaken and allowed to remain in a refrigerator at a temperature of about 10° C. for about twenty-four hours. Before being used in experiments, extracts prepared as outlined above were filtered through ordinary filter paper. In nearly all experiments referred to in the following pages about 0.1 gm. of powder of the

dried parasite in question was added to each cubic centimeter of physiological salt solution. Equal parts of freshly drawn blood and of extract were used in each experiment. Each experiment was controlled by adding to a quantity of the blood that was used in the test an equal volume of physiological salt solution.

Weinberg's conclusions concerning the presence in worms belonging to the genus Strongylus of a substance that inhibits coagulation of blood were confirmed. Extracts of *Strongylus edentatus* and of *Strongylus vulgaris* inhibited coagulation of rabbits' blood for periods ranging from thirty minutes to sixty minutes, as compared with controls. Rabbit blood in contact with extracts of specimens of *Strongylus edentatus* that had been preserved in alcohol for several weeks showed no delay in coagulation. The substance in these worms that delays coagulation of blood is evidently less potent for rabbit blood than for horse blood, which probably indicates that it has a selective action on the blood of its host. That this substance is not limited to the anterior part of the worm, as is the case in worms of the genus Ancylostoma, was shown by the following experiment:

The anterior portions (roughly about one-third of the total length of the worms) of seven dried specimens of *Strongylus edentatus* were triturated in a mortar and extracted in one cubic centimeter of physiological salt solution. The remaining posterior portions of these specimens were also triturated and extracted in an equal quantity of salt solution. Freshly drawn rabbit blood in contact with the above extracts remained uncoagulated one hour, whereas the control was coagulated in five minutes. The blood in the tube containing the extract of the posterior portion was still fluid when that in the tube containing the extract of the anterior portion was beginning to coagulate, but the difference between the rapidity of coagulation of the two samples of blood was only five minutes.

A series of experiments was performed with extracts of cattle hookworms *(Bustomum phlebotomum)*. Experiments 1 to 5 were performed with five different samples of freshly drawn cattle blood.

Experiment 1: The blood remained uncoagulated for thirty minutes. The blood in the control tube coagulated in ten minutes.

Experiment 2: The blood remained uncoagulated two and one-half hours. The blood in the control tube became coagulated in ten minutes.

Experiment 3: The blood remained uncoagulated for two and one-half hours. The blood in the control tube became coagulated in fifteen minutes.

Experiment 4: The blood remained uncoagulated three and one-half hours. The blood in the control tube became coagulated in fifteen minutes.

Experiment 5: The blood was still uncoagulated after twenty-four hours. The blood in the control tube became coagulated in ten minutes.

Experiment 6: Rabbit blood was used in this experiment. The blood remained uncoagulated for fifty minutes. The blood in the control tube was coagulated in seven minutes.

In the series of experiments upon cattle blood it was observed that only a portion of the blood actually coagulated. In the control tubes the blood clot was from two to three times as large as that in the tubes containing the extract. The latter showed a heavy sediment of erythrocytes, whereas the control tubes showed but a slight sediment of red blood corpuscles.

Experiments with extracts of a closely related species, namely, *Bustomum trigonocephalum,* a hookworm parasitic in sheep, yielded the following results: Two samples of rabbits' blood showed a delay in coagulation of twenty minutes as compared with the controls, and one sample of cattle blood showed a delay of forty-five minutes as compared with the control.

In order to determine whether the substance in the worms that inhibits coagulation of blood is readily soluble in salt solution, powder used in experiments 1 to 6 which had been extracted once was re-extracted and tested on samples of cattle blood with the following results: In two cases no effects were produced, since coagulation occurred in the controls and in the tests at the same time. In one case coagulation was delayed ten minutes as compared with the control and in another case it was delayed fifteen minutes, thus showing that the first extraction removed practically the entire anticoagulin from the parasite material.

Extracts of the stomach worm *(Haemonchus contortus)* were tested on ten samples of sheep blood, on five samples of cattle blood, and on several samples of rabbit blood. In nearly all cases the blood in contact with the extracts coagulated more slowly than the controls, but the maximum delay in coagulation of blood in contact with *Haemonchus contortus* extract as compared with the controls was about fifteen minutes.

Extracts of the kidney worm of swine *(Stephanurus dentatus),* of the lungworm of sheep *(Dictyocaulus filaria),* and of the gapeworm of poultry *(Syngamus trachealis)* were tested on three samples of sheep blood with negative results.

Extracts of *Oesophagostomum columbianum,* the nodular worm of sheep, prepared by macerating twelve fresh specimens in 2 c.c. of physiological salt solution, produced no effect on two samples of rabbit blood and one sample of cattle blood.

The negative results obtained in these experiments, as well as the weakly positive results obtained with *Haemonchus contortus,* serve as a control on the specificity of the reaction with extracts of species of Ancylostoma, Bustomum, and Strongylus, and show quite conclusively that the substance or substances in the worms which inhibit coagulation of blood are specific anticoagulins physiologically related to hirudin and certain snake venoms, and not merely mixtures of

proteins in solution. While solutions of proteoses, of trypsin, of pepsin, and extracts of tissues are known to retard coagulation of blood when injected into the living animal, they have been shown not to delay coagulation of blood *in vitro,* and hence the effects on coagulation of blood *in vitro* produced by extracts of certain nematodes cannot be ascribed to such substances.

A series of experiments with *Ascaris lumbricoides* fluid and rabbit blood yielded the following results: Three to five drops of fluid of *Ascaris lumbricoides* from swine in contact with ten drops of blood produced a delay in coagulation of fifteen minutes as compared with the control. A mixture of eight drops of fluid and ten drops of blood remained uncoagulated thirty-five minutes longer than the control. A mixture of ten drops of fluid and ten drops of blood showed a delayed coagulation of forty-two minutes as compared with the control.

Extracts of *Ascaris equorum* and of *Belascaris* sp. were tested on three samples of sheep blood with weakly positive results, i. e., blood in contact with these extracts remained uncoagulated five to fifteen minutes longer than the controls.

These experiments confirm Flury's results with human blood and dog blood and show that the fluid that is present in the body cavity of *Ascaris lumbricoides* delays to a certain extent coagulation of blood *in vitro.*

DISCUSSION

That certain nematodes secrete substances that have toxic properties and that are absorbed by the host is a view which has been advanced by a number of investigators. In addition to the toxic principle discussed in this paper, other specific toxic substances, especially hemolysins, have been shown to occur in several species. It is probable that the toxic secretions of nematodes, like snake venoms, are a complex of a number of different principles, such as hemolysins, anticoagulins, and one or more systemic poisons.

It is of interest to note that nematodes that contain anticoagulins also contain hemolysins.[3] The former are probably distinct from the latter chemically as well as physiologically. The hemolysin found in species of Ancylostoma is destroyed by heating at 55° C. for several minutes (Whipple, 1909; Schwartz, 1920), whereas the anti-coagulin in these worms resists boiling (Loeb and Smith, 1904). Further studies on the properties of anticoagulins from nematodes would probably yield interesting comparisons with those of hemolysins. Investigations concerning the possible presence of hemolysins in nematodes that lack anticoagulins would also be of interest.

3. A brief account of hemolysins from parasitic worms is given in a recent paper by the writer (Schwartz, 1920).

It is conceivable and by no means improbable that substances in nematodes which delay coagulation of blood may be of etiological significance in the pathology of nematode diseases. Loeb and his collaborators are inclined to the view that the oozing of blood from the wounds inflicted by hookworms, rather than the absorption by the host of a hemolysin elaborated by the parasites, accounts for the anemia that occurs in cases of infestation with these parasites. That the anticoagulin which has been shown to occur in the anterior portion of these parasites is responsible for the persistent hemorrhages in question appears to be a warranted conclusion. In this connection it is of interest to observe that markedly strong anticoagulins, so far as is known, occur only in nematodes belonging to the family Strongylidae, the members of which commonly produce pronounced anemia in the host.

SUMMARY

1. The substance in species of Strongylus that inhibits coagulation of blood is present in the posterior as well as in the anterior portion of the worms.

2. *Bustomum phlebotomum,* a hookworm parasitic in cattle, contains a substance soluble in salt solution that inhibits coagulation of blood for considerable periods which vary with different samples of blood. A closely related species, *Bustomum trigonocephalum,* contains a similar anticoagulin.

3. Salt solution extracts of *Haemonchus contortus* cause but a slight delay in coagulation of blood. Extracts of *Syngamus trachealis, Dictyocaulus filaria,* and *Stephanurus dentatus* do not retard coagulation of sheep blood. Extracts of *Oesophagostomum columbianum* do not retard coagulation of rabbits' blood.

4. The body fluid of *Ascaris lumbricoides* inhibits coagulation of blood to a moderate extent. Extracts of *Ascaris equorum* and of *Belascaris* sp. have a slight effect on coagulation of sheep's blood.

5. In view of the fact that the delay in coagulation of blood due to extracts of nematodes occurs *in vitro,* that it varies with extracts of different species of worms, and that extracts of certain species produce no delay in coagulation, it may be concluded that specific substances, other than proteins in solution, must be involved.

6. The substances in question appear to be physiologically related to hirudin and snake venom, and like the latter are probably part of a complex of toxic principles.

7. So far as present knowledge goes, nematodes which contain substances that inhibit coagulation of blood to a marked degree are zoologically related, belonging to the family Strongylidae, the mem-

bers of which have a buccal capsule adapted to lacerating the intestinal mucosa.

8. That the injection of their secretions into the intestinal mucosa, by certain biting nematodes, resulting in minute hemorrhages, is of etiological importance in nematode diseases appears very probable.

References Cited

Flury, Ferdinand. 1912.—Zur Chemie und Toxikologie der Ascariden. Arch. exper. Path. u. Pharmakol., 67: 275-392.

Leroy, Alphonse. 1910.—Sur la toxicité du liquide périentérique d' "*Ascaris megalocephala*." Arch. internat. physiol., 9: 276-282.

Liefmann, H. 1905.—Beitrag zum Studium der Ankylostomiasis. Ueber den Infectionsmodus und die vermuthliche Giftwirkung der Würmer. Ztschr. Hyg. u. Infectionskrankh., 50: 349-363.

Loeb, Leo. 1906.—Ein weiterer Versuch über die die Blutgerinnung hemmende Substanz in *Ankylostoma caninum*. Centralbl. Bakteriol., 1. Abt., Orig., 40: 740-741.

Loeb, Leo, and Fleisher, M. S. 1910.—The influence of extracts of *Anchylostoma caninum* on the coagulation of the blood and on hemolysis. J. Infect. Dis., 7: 625-631.

Loeb, Leo, and Smith, A. J. 1904.—The presence of a substance inhibiting the coagulation of the blood in *Anchylostoma*. Proc. Path. Soc. Phila., n. s., 7: 173-178.

1904 a.—Ueber eine die Blutgerinnung hemmende Substanz in *Anchylostoma caninum*. Centralbl Bakteriol., 1. Abt., Orig., 37: 93-98.

Schwartz, Benjamin. 1920.—Hemolysins from parasitic worms. Arch. Int. Med., 26: 431-435.

Weil, P. Emile, and Boyé, G. 1910.—Action des extraits d'*Ascaris equorum* sur la coagulation du sang de lapin. Compt. rend. soc. biol., 69: 284-285.

Weinberg, M. 1907.—Sur une hémotoxine d'origine vermineuse. Compt. rend. soc. biol., 63: 13-15. Also in Rev. vét., 64 [n. s.] 32: 701.

Whipple, G. H. 1909.—The presence of a weak hemolysin in the hook worm and its relation to the anemia of uncinariasis. J. Exper. Med., 11: 331-343.

A MICROSPORIDIAN OCCURRING IN THE SMELT

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In the course of taxonomical studies on the smelts, Dr. A. C. Kendall and D. R. Crawford of the Bureau of Fisheries have frequently encountered a very characteristic infection in these fishes. This infection I find to be caused by one of the Microsporidia, belonging to the genus Glugea.

Infections at various stages of development were available. Apparently, the intestine is the primary seat of the parasite. Affected fishes are characterized by the appearance of more or less numerous cysts in the viscera, and generally all the cysts in a fish are of approximately the same size. The latter may vary from microscopical dimensions to 3 mm. in diameter. Early stages show them in the wall of the intestine where their white color makes them conspicuous even when still small. At this time they are located in the mucosa, below the epithelium of the villi (Fig. 2). As growth proceeds, they push through the muscular coat of the intestine, and then come to lie immediately under the peritoneum. An extreme but common manifestation of such cyst development is shown in Figure 1. The entire length of the intestine from below the stomach to within a short distance of the anus is here taken up with cysts, and it becomes a puzzle how it can function under such conditions. Cysts also occur in the liver and the gonads, but not one was found in the stomach proper, kidney or heart.

The distribution of affected smelts is a very interesting one. Such fishes were found in Lake Massabesic, N. H.; Sunapee Lake, N. H., near Dennysville on the extreme northern portion of the Maine coast, and Casco Bay on the southern Maine coast. The smelts in Sunapee Lake were introduced some years ago from Squam Lake, N. H., from which no records are available. It is to be noted that the specimens from the Maine coast are typical smelts which live in salt water and ascend fresh water streams at a certain time each year. Those from the lakes mentioned, however, are purely fresh water forms and never come in contact with salt water. Aside from other considerations, dams and other obstructions would make journeys from the sea into these lakes a physical impossibility. It may also be remarked that Dr. Kendall (paper in preparation) believes that these fresh water smelts are taxonomically distinct from the salt water form, *Osmerus mordax*.

Nevertheless, the Microsporidian parasites seem to be identical in both fresh and salt water smelts. Whether the parasite became estab-

lished after the several types of smelts had evolved, or whether it was present originally and underwent no morphologic changes in distinction to its host, must be left unanswered at present.

The rate of infection may be very high, as is manifested by the following data:

Lake Massabesic, N. H.: 38 out of 71 infected—over 53%. (Mild infections were not counted in this instance.)

Sunapee Lake, N. H.: 29 out of 103 infected—over 28%.

Dennysville, Me.: 1 out of 64 infected—over 1.5%.

Casco Bay, Me.: 48 out of 306 infected—over 16%.

In Casco Bay the distribution is very general, and affected fishes were caught at Mosiers Island and Freeport (Harraseeket River, Mast Landing Creek, and Porters Landing Creek). Collections were made in 1904, 1907, and 1915, and generally either in spring or autumn.

Adult fishes are only rarely parasitized, the highest rate of infection being found among immature fishes of approximately 10 cm. in length (generally speaking, about a year before maturity). Fishes of the latter size are very much more numerous than adults. Possibly the scarcity of parasitized adults is due to the fact that the majority of infected immature fishes die, leaving only those that escape infection to attain maturity. It must not be forgotten, of course, that such a disparity in the numbers of young and mature fishes is encountered to some degree in all other species of fishes, where parasitism may not be instrumental at all.

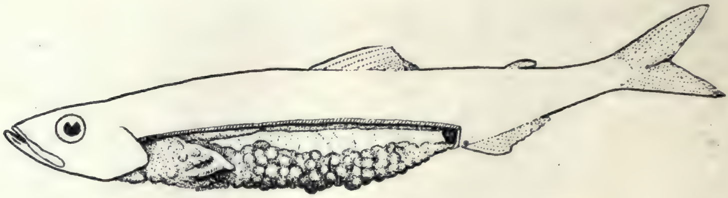
Glugea sp.

Cysts vary in size from microscopic dimensions to 2 or 3 mm. in diameter. As in other species of Glugea, sporonts, sporoblasts, and ripe spores may all be found in a single cyst, with the earliest stages near the periphery. Spore formation seems to follow the same lines as described for *Glugea anomala* by Stempell (1904), and Awerinzew and Fermor (1911).

Dimensions of spores: length $= 4$ to 4.5μ; width $= 2$ to 2.5μ.

The preservation of the material rendered a study of the nuclear conditions in the spores impossible, although other developmental stages were not badly fixed. The giant vegetative nuclei which have been the basis of much dispute, are very numerous at the periphery of developing cysts. To all appearances, they give rise to sporonts, as Stempell and Awerinzew and Fermor have maintained, and are not hypertrophied tissue nuclei of the host (Schuberg, 1910, and Schroeder, 1909).

Unlike *Glugea anomala,* the parasite is specific for the smelts and does not affect even other Salmonidae inhabiting the same waters. (It is indeed open to question whether the Microsporidia of Gasterosteus could be transmitted to Gobius, although Stempell believes that in both species of fishes, which are of widely different families, *Glugea anomala* is concerned.) Again, the size given above is fairly constant, and no such extreme variation as described in *G. anomala* could be



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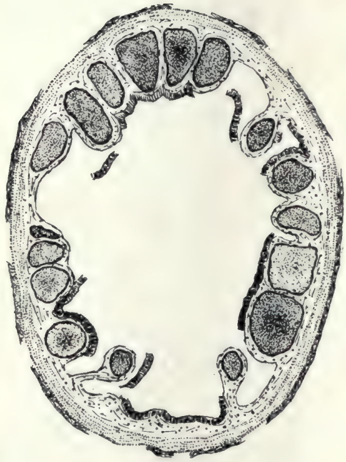

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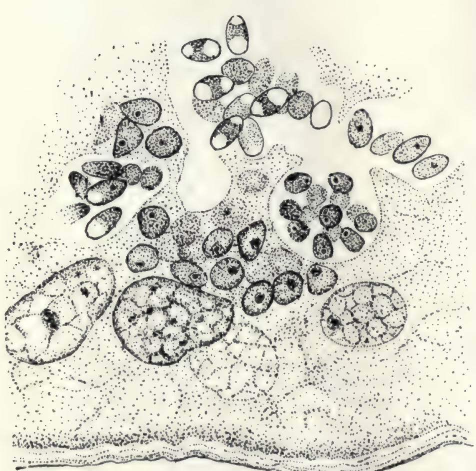

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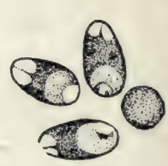

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PLATE XIV

observed. Muscles and connective tissue were found not to be subject to invasion, which also is a point of difference from that described species.

Microspore infections of the smelt have also been reported for North America by Mavor (1915) and Linton (1901). The latter records sporocysts in the intestine of this fish, but does not go into detail any more than Mavor, who contents himself with the statement that the microspore concerned in his case was apparently *Glugea stephani.* The measurements of the latter, which is typically a parasite of the flatfishes, differ definitely from the parasite which I have described—3 by 1.5 mm. (Johnstone, 1901). Its mode of occurrence in the intestine and its life history are, however, markedly similar, and it seems probable that both Mavor and Linton were dealing with the same parasite as that discussed in this paper.

A form which in both size and occurrence corresponds almost exactly to the one I have described, was found by Weissenberg in the European smelt, *Osmerus eperlanus,* and named *Glugea hertwigi.* The dimensions given for this form are 4.6 to 5.4 by 2.3 μ, which measurements are very close to those of the American form. Weissenberg's measurements were taken from fresh specimen, which may account for their slightly larger proportions. The occurrence of the same parasite in European and American species of the smelt would furnish an interesting parallel to the case of *Myxidium lieberkuehni* which according to Mavor occurs in both the European and the American pike.

References Cited

Awerizew, S., and Femor, K. 1911.—Studien über parasitische Protozoen. Zur Frage über die Sporenbildung bei Glugea anomala. Arch. Protist., 23: 1-6.

Johnstone, J. 1901.—Note on a Sporozoon Parasite of the Plaice (Pleuronectes platessa). Proc. Trans. Liverpool Biol. Soc., 15: 184-187.

Linton, E. 1901.—Fish Parasites collected at Woods Hole in 1898. Bull. U. S. Fish Com., 19: 267-304.

Mavor, J. W. 1915.—Studies on the Sporozoa of the Fishes of the St. Andrew's Region. Contrib. Canad. Biol., paper 39 b: 25-38.

Schröder, O. 1909.—Thelohania chaetogastris, eine neue in Chaetogaster diaphanus Gruith schmarotzende Microsporidienart. Arch. Protist., 14: 119-133.

Schuberg, A. 1910.—Ueber Microsporidien aus dem Hoden der Barbe und durch sie verursachte Hypertrophie der Kerne. Arb. Kais. Gesund., 33: 401-434.

Stempell, W. 1904.—Ueber Nosema anomalum Monz. Arch. Protist., 4: 1-42.

Explanation of Plate

1. Smelt showing advanced infection of liver and intestine. Reduced one fifth.

2. Cross section of intestine showing an early infection. 20 ×.

3. Portion of parasitized testis. Objective: 32 mm. Eyepiece: 6 ×.

4. Periphery of developing cyst showing giant nuclei and developmental stages. Objective: 1.5 mm. Eyepiece: 10 ×.

5. Spores. Objective: 1.5 mm. Eyepiece: 15 ×.

THE FIRST INSTAR OF *WOHLFAHRTIA VIGIL* WALKER

O. A. Johannsen

In the September number of this journal Dr. E. M. Walker (1920) published an account of the larval structure and habits of *Wohlfahrtia vigil.* As the first instar has not yet been described the following may be of interest:

On July 9, 1907, in Ithaca, N. Y., the writer captured a female specimen, which when placed in the cyanide bottle, immediately deposited several larvae on the side of the glass. The species is therefore larviparous, like other Sarcophagids. As the adult of this species has several striking characteristics, it was possible, even at that date, and before the appearance of Aldrich's monograph (1916) to determine it by means of the original description of Francis Walker (1849), under the name of *Sarcophaga vigil.* Though not common in Ithaca, I have taken a few specimens of the fly nearly every season since 1907, and always in a similar situation, that is, on a cement walk, in the bright sunshine, about midday, during June, July and August.

The larva of the first instar measures 2.3 mm. in length, by 0.4 mm. in width in the region of the fifth or sixth abdominal segment. The upper pair of tubercles (or antennae of Portchinsky) of the pseudocephalon, are well defined, 30μ in length, two-segmented, the first segment about as long as broad, the second somewhat longer than broad, and conical. The mandibular hooks are slender, sharply pointed, and much curved, though less so than those figured for *Wohlfahrtia magnifica* Schiner by Portchinsky (1884). He states that in the first instar of the last mentioned species there is a large median hook placed a little higher than the laterals. In *W. vigil* there is no indication of this median hook. As it is scarcely conceivable that so experienced an observer as Portchinsky should mistake a portion of the pharyngeal skeleton for a median hook, we must conclude that *W. magnifica* differs from the American species in this particular. As in the first instar of the house fly larva the anterior spiracular processes are lacking. The posterior spiracles are in a pit and each has two slits which lie parallel to each other, approximately perpendicular to the horizontal plane, supposing the larva to be lying ventral side down in a horizontal position. The spinules on the body are rather larger and more numerous than indicated by Walker (1920) for the second and third instars, though less widely distributed than described by Portchinsky (1875) for *W. magnifica.* In my specimens the anterior third of the second

segment, and the anterior fourth of the third, fourth and fifth segments are each provided with a uniform spinule band. On the succeeding segments the bands occupy about one-fourth the width of a segment, are placed over the incisures and all are more or less interrupted by clear, transverse areas, corresponding to the folding at the incisures. The incisure cuts the fifth segment near its anterior margin, but the bands at the posterior end of the body are cut by the incisures nearer their middle. The clear areas are more numerous in the posterior bands, but their arrangement does not appear to be significant since this differs in distribution in different specimens. The spinules in the bands at the cephalic end are somewhat larger than those in the posterior bands.

The first instar, on the whole, is therefore more distinctly spinose than the second or third, as illustrated by Dr. Walker, for this species, but is less spinose than the European species described and figured by Portchinsky.

REFERENCES

Aldrich, J. M. 1916.—Sarcophaga and its Allies in North America. The Thomas Say Foundation.

Portchinsky, J. 1875.—Krankheiten, welche im Mohilew'schen Gouvernement von den Larven des *S. Wohlfahrti* entstehen, und deren Biologie. Horae Soc. Ent. Ross., 11 : 122.

1884.—*Sarcophila wohlfahrti.* Horae Soc. Ent. Ross., 18 : 247.

Walker, E. M. 1920.—*Wohlfahrtia vigil* (Walker) as a human parasite. Jour. Parasitol., 7 : 1.

Walker, Francis. 1849.—List of the Dipterous Insects. See *Sarcophaga vigil,* part 4, page 831.

NOTES

A NOTE ON LONGEVITY OF LARVAL TICKS

Some time about the last week of July, 1919, a small boy living at our Biological Station brought me a number of engorged ticks which he had taken from the ears of his dog. I placed two of the largest in a small wooden box with a tight cover. In two or three days they began laying eggs. This process continued for several days when the ticks and about half the eggs were taken out of the box. Some time near the first week in August, the eggs began hatching and all were hatched by about the middle of August. There were then several hundred newly hatched ticks in the box ready and waiting to be fed, but I carefully refused to be the victim.

The box was then left closed until November 14, 1919, when it was opened and a large number were still alive and active. In fact their numbers and activity prevented the making of any estimate as to how many were actually living. On December 28, 1919, the living larvae were still numerous as was also the case on January 4, 1920. By February 25, 1920, the numbers were so reduced and the activity so diminished that it was possible to estimate fairly satisfactorily that about fifty still lived. On March 11, 1920, only a few were alive and they were rather inactive. On April 9, 1920, none could be found showing signs of life.

The dozen or so which survived this period of seven months without ever having fed show very clearly that the perpetuation of this species is well provided for by qualities of endurance as well as by great numbers of young. One is led to wonder what changes occurred in form and function of organs of these animals during the ordeal. In some respects the tick would appear to be an organism unusually favorable for certain studies in nutrition.

Read at the Western Society of Naturalists, Seattle, June, 1920.

W. E. Allen.

Professor Ludwig von Graff died in Graz on December 8, 1920. While his work had been almost entirely in other fields he had written a small but valuable book on the parasites of domestic animals transferable to man and a large and extremely valuable work on The Turbellaria as Parasites and Hosts. The death of Professor Graff removes a commanding figure from the field of zoological research.

The library of the late Doctor A. J. Chalmers has been presented to the Royal Society of Medicine (England). It is to be known as the Chalmers Collection and to constitute the library of the new Section of Tropical Medicine and Parasitology. The British Medical Journal states that this is probably the finest collection of books on tropical medicine to be found anywhere.

Dr. Benjamin Schwartz has resigned as Assistant Zoologist in the Bureau of Animal Industry and has accepted the position of Professor of Protozoology and Parasitology in the University of the Philippines at Manila.

Dr. E. C. Faust has been given editorial charge of the department of Parasitology in the China Medical *Journal*.

ERRATA

In The Journal (June, 1920). vol. VI, p. 175, line 4 from bottom, for 31 (the number of hooks) read 33.

In The Journal (December, 1920), vol. VII, p. 63, line 2, for *Grahamella protista* read *Grahamella talpae* n. g., n. sp. of Protista (Brumpt, 1911:514).

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CYTAMOEBA BACTERIFERA IN THE RED BLOOD CELLS OF THE FROG

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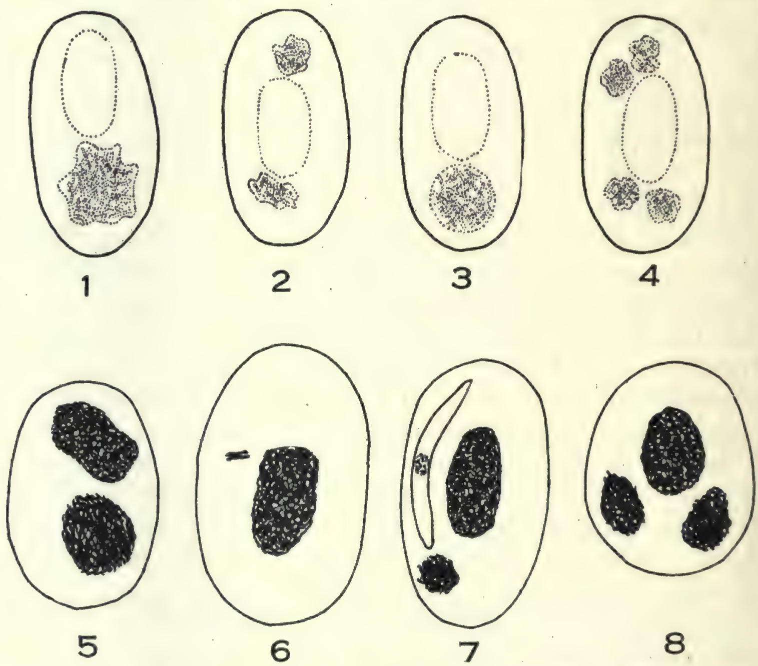
This note is intended to call to the attention of parasitologists and bacteriologists the presence in the red blood cells of frogs in this country of a most interesting organism to which the name *Cytamoeba bacterifera* was given by Labbé in 1894.

Cytamoeba bacterifera was found in the red cells in the peripheral blood of two specimens of *Rana clamitans* and five specimens of *R. catesbiana* collected at Cold Spring Harbor, Long Island, N. Y., in the summer of 1919. It was absent from the other frogs and toads examined; namely, *Rana palustris, R. sylvatica, R. pipiens, Hyla versicolor, Bufo americanus* and *Scaphiopus holbrookii.* In every case the host was also parasitized with trypanosomes, hemogregarines, and several other forms that represent either undescribed species or stages in the life cycles of parasites known only as adults. Freshly drawn blood was studied as well as material fixed and stained by Wright's method and by the Schaudinn-iron-haematoxylin method. The frogs were kept alive in the laboratory for a considerable period and their blood examined at intervals of a few days; several of them were kept in this way for three months.

The importance of studying specimens of Cytamoeba while alive and in freshly drawn blood was soon demonstrated, since movement ceases shortly after being taken from the frog's body and the appearance in stained preparations is far from normal. A typical, fully grown specimen as seen when alive and active is shown in figure 1. It measures about 7μ in diameter and occupies a position at one end of the red cell, sometimes pushing the nucleus out of its usual central position. The entire parasite is active as well as the bacilliform bodies within it. It resembles very much a trophozoite of the tertian malarial parasite, *Plasmodium vivax,* throwing out and withdrawing pseudopodia on all sides. At the same time the bacilliform bodies are moving about rapidly within, creating a flame-like appearance. After a few minutes the

movements of the parasite cease and a stationary, rounded shape is assumed (Fig. 3), but the bacilliform bodies continue to move about within, often for several hours. Finally all movement of both parasite and bacilliform bodies ceases. Many stages in the growth of the parasite are present within the red blood cells of a frog at the same time. The first recognizable stages appear like minute vacuoles frequently situated at the side of the nucleus of the red cell instead of at one end as later. Only one or a few bacilliform bodies may be present at this time (Fig. 6). As growth proceeds the size of the Cytamoeba increases as well as the number of bacilliform bodies. Fully grown parasites differ with respect to the number of these bodies, some being supplied



All figures were drawn with a camera lucida × 1440.

Fig. 1. Large, living, active specimen of *Cytamoeba bacterifera* within red blood cell of bullfrog. The nucleus of the blood cell is pushed out of its normal position. The parasite has thrown out pseudopodia and is filled with active bacilliform bodies.

Fig. 2. Red blood cell containing two living, active specimens.

Fig. 3. Red blood cell containing one living specimen that has rounded up and has ceased to throw out pseudopodia. The bacilliform bodies within are still active.

Fig. 4. Red blood cell containing four living, active specimens.

Fig. 5. Red blood cell containing a large specimen. This and figures 6, 7 and 8 were drawn from material fixed and stained by Wright's method.

Fig. 6. Red blood cell containing a very young specimen with only two bacilliform bodies.

Fig. 7. Red blood cell infected with both Cytamoeba and Haemogregarina.

Fig. 8. Red blood cell containing two specimens of Cytamoeba.

with more than others. Blood specimens from frogs that were kept in the laboratory were examined at intervals for three months but no other stages in the life history of the Cytamoeba were found and no great differences were observed in the percentage of infection of red blood cells or in the sizes of the organisms present. For example, in a bull frog (No. 23) the percentage of infection of red blood cells ranged from 4 to 7 at different times during a period of three months. The infection in other specimens ranged from 1% up to 12%. In a few cases red blood cells contained two Cytamoebas (Figs. 2 and 8) and in one case four were present (Fig. 4). Several cells were infected with both Cytamoeba and Haemogregarina (Fig. 7). No visible difference was observed between the size and contents of the infected and noninfected red cells either in fresh blood or in the fixed and stained material such as is sometimes so conspicuous when cells are parasitized by large specimens of Haemogregarina or Karyolysus. No specimens of Cytamoeba were found free in the blood as reported by Labbé (1894) and Laveran (1899).

Specimens fixed and stained by Wright's method present an entirely erroneous appearance. The amoeboid or rounded shape of the parasite is entirely lost due probably to drying and an irregular mass of bacilliform bodies is left. These stain deeply and are quite conspicuous. The appearance of the parasite and its contained bodies is indicated in the accompanying figures (Figs. 5-8). Specimens fixed in Schaudinn's solution and stained with iron-haematoxylin are unsatisfactory. They keep their rounded shape, but do not stain well.

Several interesting questions regarding Cytamoeba remain to be solved. Is Cytamoeba an organism or only a vacuole in which bacteria live? If it is an organism is it an "adult" stage or a developmental stage of an unknown species or of a known species such as *Haemogregarina ranarum* or *Trypanosoma rotatorium*? If it is a stage of an unknown species, what stage in the life cycle does it represent and in what part of the body do the other stages occur? Are the bacilliform bodies bacteria? If so, how does the Cytamoeba become infected by them?

Kruse (1890), probably the first to figure this parasite, speaks of it not as an organism but as a space in the blood. Gabritchewsky's (1890) attention was called to it by Metchnikoff who found it in the blood of frogs infected with *Haemogregarina ranarum*. His efforts were chiefly directed toward an investigation of the "bacteria," nevertheless the "petite boule protoplasmique transparente" which contained these bacteria was considered "un être complètement, étrange à l'hématie, une espèce d'amibe, ou de larve amibiforme envahie elle-même par un microbe bactérien." Four uninfected frogs were inoculated with the spleen, liver, kidney and bone marrow of a frog con-

taining both Haemogregarina and Cytamoeba. Three of the frogs were later found to possess both types of parasites; in the fourth animal only the Cytamoebae were present. Autopsies revealed the Cytamoebae in the spleen, and bone marrow. A small number of trypanosomes were noted in all frogs containing the other parasites. Culture experiments gave negative results. Gabritchewsky suggests that perhaps "le corpuscle amibiforme" is a stage in the development of Haemogregarina.

The name *Cytamoeba bacterifera* we owe to Labbé (1891, 1894). This investigator recognized it as a new type of organism and placed it in the genus Cytamoeba, a generic name proposed previously by Danilewsky (1890) for the malarial parasite. A parasite of the red blood cells of *Hyla viridis* described by Grassi (1882) and considered by him analogous to the malarial organism was placed by Labbé provisionally in the same genus and given the name *Cytamoeba grassi*. Labbé describes what he thought were stages of Cytamoeba in fission and spore formation, and specimens that he found free in the blood.

Wasielewski (1896) accepted Labbé's views regarding the protozoan affinities of *Cytamoeba bacterifera* and placed it in the order Acystosporidia, family Haemamoebidae, along with the genera Proteosoma, Haemamoeba, and Dactylosoma. Cytamoeba was next described by Ziemann (1898) who noted the amoeboid movement of vacuole-like bodies within the red blood cells of the frog, and observed the bacilliform bodies contained in them but was not convinced that the body he saw was really an amoeboid organism.

Laveran (1899) also observed *Cytamoeba bacterifera* both in fresh and in stained preparations. He named the bacilliform bodies *Bacillus krusei* and concluded that the bacteria were not contained in a parasitic ameboid organism, because (1) no movement of the vacuole was observed; (2) the bacilliform bodies moved actively as though in a liquid and not in protoplasm; (3) isolated bacilli were encountered, without a surrounding clear area; (4) the vacuoles did not become stained and no nucleus could be found; (5) after the dissolution of the protoplasm of the red blood cell, the bacteria were isolated; and (6) no reproduction of the "ameba" was observed. Laveran did not succeed in cultivating *Bacillus krusei*. Several of Laveran's contentions do not hold since (1) the "vacuole" certainly undergoes amoeboid movement, (2) protoplasm within certain amoebae is liquid as is probably also true of Cytamoeba and the active movement of the bacilliform bodies is possible in this medium, (3) there is no reason why bacilli should not exist outside of the organism, (4) failure to find a nucleus does not prove that one is not present since it may be obscured by the bacilliform bodies, (5) the protoplasm of the organism as well as that of the red cell may have been dissolved, and (6) the reproduction of the "ameba" may occur in the internal organs.

More recently Dutton, Todd and Tobey (1907) have described several organisms that were found by them in the red blood cells of frogs in Africa, some of which may have been Cytamoebas; and Carini (1910) has attempted to classify similar bodies found in the Brazilian frog, *Leptodactylus ocellatus*. These were apparently observed only in stained specimens and hence were unsatisfactory for study. Certain of them are called by Carini "elements bacilloides"; a second type consists of bacilliform bodies similar to Bacillus krusei but thinner and shorter, and a third type resembles Cytamoeba.

After studying infected red blood cells of the frog both living and fixed and stained and reviewing the literature on this subject the tentative conclusion is reached that *Cytamoeba bacterifera* is a stage in the life cycle of a protozoan parasite and that living within it either as hyperparasites or in symbiosis is a bacillus named by Laveran *Bacillus krusei*. The writer hopes at some future time to add more to our knowledge of this interesting organism.

Literature Cited

Carini, A. 1910.—Sur quelques parasites semblables a des bacilles rencontres dans les hématies du Leptodactylus ocellatus. Ann. Inst. Pasteur, 24:1-5; 2 pl.

Dutton, Todd and Tobey. 1907.—Concerning Certain Parasitic Protozoa Observed in Africa. Ann. Trop. Med and Parasit., 1:334.

Gabritchewsky. 1890.—Contribution a l'etude de la parasitologie du sang. Ann. Inst. Pasteur, 4:440-445.

Kruse, W. 1890.—Ueber Blutparasiten. Archiv. path. Anat. Phys., 120:541-560.

Labbé, A. 1894.—Recherches zoologiques et biologiques sur les parasites endoglobulaires du sang des vertébrés. Théses. Paris, 208 pp., 10 pl.

Laveran, A. 1899.—Sur le bacille parasite des hématies de Rana esculenta. Compt. rend soc. biol., 51:355-358.

Wasielewski, T. von. 1896.—Sporozoenkunde. 162 pp., 111 figs. Jena.

Ziemann, H. 1898.—Ueber Malaria und andere Blutparasiten. 191 pp., 5 pl. Jena.

TWO NEW MONOSTOMES FROM ASIA *

E. C. Harrah

Although monostomes have been widely studied only a single specimen has been reported from Asia viz., that described by Stossich (1902) as *Haematotrephus phaneropsolus,* from Totanus sp. taken at Yedo, Japan. Previous to that the eastern limits of the group were represented by *Cyclocoelum nigropunctatum* (von Linstow 1883) in Turkestan and *Cyclocoelum tringae* (Brandes 1892) from *Tringa variabilis* taken on the penninsula of Sinai. Within the last decade Skriabin (1913) described *Cyclocoelum orientale* from *Totanus glareolus,* and *Tracheophilus sisowi* from *Anas boschas,* taken in Russian Turkestan and *Catatropis charadii* from *Helodromus ochropus* taken along the Ural mountains.

Nicoll (1914) and S. J. Johnston (1916) reported a number of species taken from birds in Australia. Nicoll called attention to the fact that except for *Allopyge antigones* the forms found were not particularly Australian in character, while Johnston demonstrated a direct relationship between *Cyclocoelum taxorchis* and a Brazilian species.

The material which forms the basis of this paper is composed of two species, one from a magpie, probably *Cyanopolius cyanus,* taken at Nanking, China, by R. T. Shields in 1915; the other from the fantail snipe *Gallinago gallinago* (L) taken at Chiengmai, Siam, by M. E. Barnes, in January, 1918. For the loan of this material the writer is deeply indebted to Professor Henry B. Ward, to whom the above collections were sent, and wishes to take this opportunity to express to Professor Ward his sincere thanks for many valuable suggestions during the progress of this work as well as for the loan of the material.

Cyclocoelum elongatum nov. spec. [Fig. 1]

Narrow elongate monostomes 12 to 16.5 mm. in length by 1.5 to 3 mm. in greatest width which is found a little posterior to the middle of the body. From this point the body tapers toward both ends which are bluntly rounded and approximately equal in size. The subterminal mouth opening is surrounded by a well developed spherical sucker, measuring 430 to 520μ in diameter, which is closely followed by a much smaller ovoid pharynx, 215 to 280μ wide by 265 by 330μ long. The esophagus is of moderate length. The genital pore is situated ventral to the posterior end of the pharynx. The cirrus pouch stretches from this point to the posterior wall of the intestinal bifurcation. The vitelline glands extend from the region of the pharynx to the excretory bladder at which place they frequently overlap; in lateral extent they rarely reach over the outer intestinal wall. The excretory bladder is bicornuate and in this respect *Cyclocoelum elongatum* differs from all other species of this genus.

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* Contribution from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 183.

The genital glands are intracecal in position, the posterior testis filling entirely the space of the posterior intestinal arch. It is usually flattened laterally and measures 415 to 975μ in length by 415 to 570μ in width. The anterior testis which is situated a short distance from the posterior and lateral is only a little smaller; it shows a length of 640 to 820μ and a width of 480 to 545μ. The spherical ovary which lies about equally distant from the two testes is approximately two-thirds the size of the anterior testis and measures 330 to

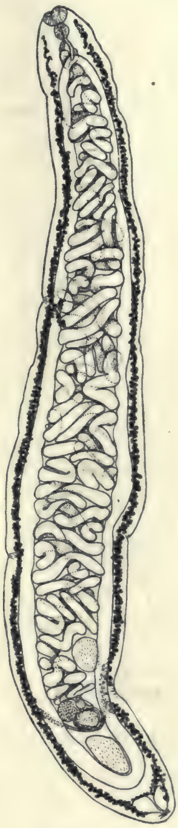


Figure 1

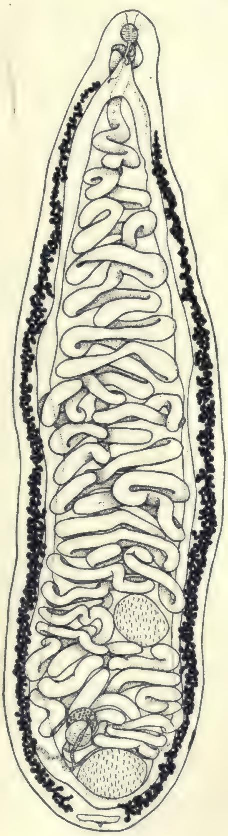


Figure 2

EXPLANATION OF FIGURES

Fig. 1.—*Cyclocoelum elongatum.* $\times$ 8.
Fig. 2.—*Cyclocoelum obliquum.* $\times$ 13.

375μ in diameter. The spherical shell gland lies dorsal and posterior to the ovary and is approximately equal in size to the latter organ. The receptaculum seminis, which lies median and dorsal to the ovary is a little less than one-half its size, measuring 165μ in diameter. In general the uterus does not pass posterior to the shell gland and anterior to that organ fills out the entire space between the intestinal crura. The eggs are thick shelled, oval, 112 to 117μ in length by 51 to 66μ in width.

Habitat: *Cynopolius cyanus* (?), Nanking, China. Organ not designated.

Cyclocoelum elongatum differs from all other known species of this genus in that it has a strong well developed oral sucker and a bicornuate excretory bladder. It is more slender than *Cyclocoelum problematicum* Stossich which species it simulates in lateral extent of the uterus.

Cyclocoelum obliquum nov. spec. [Fig. 2]

Medium sized worms, 10 mm. long by 2.5 mm. wide, in maximum which is found in the middle of the body; from this point the body tapers anteriorly to the relatively small rounded anterior end. Posterior to the point of greatest width the margins of the body are nearly parallel; the posterior end being broadly rounded. An oral sucker is present but weakly developed, 410μ in diameter; and is twice the size of the pharynx which is spherical and measures 230μ in diameter. The relatively short esophagus is approximately twice the length of the pharynx. The genital pore is situated ventral to the posterior end of the pharynx from which the cirrus pouch extends posteriorly to the middle region of the intestinal bifurcation. The uterus is loosely folded, filling out the space between the crura and passing laterad to the outer wall of the latter organ. The vitellaria extends from the posterior wall of the intestinal bifurcation almost to the excretory bladder in the posterior end of the body where the right and left halves are separated by a distinct interval. One half of the vitellaria usually extends further cephalad than the other. In a lateral direction the vitellaria rarely extend over the outer wall of the crura. The testes are unequal in size; the anterior, smaller and spherical, measures 612μ in diameter, while the posterior one is larger, slightly flattened antero-posteriorly and fills out the posterior cecal arch. It measures 660μ in length by 800μ in width. The testes are usually separated by a number of uterine loops. The spherical ovary, which is much smaller than the testes, being only 285μ in diameter, is separated from the anterior testes by a number of uterine loops and from the posterior one only by the receptaculum seminis uterinum. The shell gland, which is approximately equal in size to the ovary, is situated dorsal and posterior to that organ and measures 295μ in diameter. The small spherical receptaculum seminis, 115μ in diameter, is dorsal and median in position to the ovary. The eggs are oval, of medium size, thick shelled, and measure 122 to 127μ in length by 61 to 66μ in width.

Habitat: Liver, *Gallinago gallinago* (L), Chiengmai, Siam.

The forms described above show so little that is distinctly different from species previously described for the genus Cyclocoelum that the writer feels justified in placing them in it. *Cyclocoelum elongatum* differs from all other known species of the genus in that it has a strong, well developed oral sucker and a double excretory bladder. With respect to the lateral extent of the uterine loops and in the position of the genital glands it closely resembles *Cyclocoelum problematicum.* However, in the distribution of the vitellaria it simulates *Cyclocoelum brazilianum* Stossich. In distribution of the vitellaria and uterine loops *Cyclocoelum obliquum* is quite similar to *Cyclocoelum vicarium* (Arnsd.) while the position of the genital glands simulate that of *Cyclocoelum mutabile* (Zed.); in that the testes are unequal and the posterior also flattened, they are more like *Cyclocoelum brazilianum* than *Cyclocoelum mutabile.*

SUMMARY

1. The occurrence of the genus Cyclocoelum is established in Asia, in addition to its presence in a new host, probably *Cyanopolius cyanus*.

2. Two new species, *Cyclocoelum elongatum* and *Cyclocoelum obliquum* are described.

3. The two new species show closer relations to the European than to the Australian species of Cyclocoelum.

REFERENCES CITED

Johnston, S. J. 1916.—On the Trematodes of Australian Birds. Jour. Proc. Roy. Soc. N. S. Wales, 50: 187-261.

Linstow, O. von. 1883.—Nematoden, Trematoden und Acanthocephalen, gesammelt von Prof. Fedtschenko in Turkestan. Arch. Naturg., 49: 274-313.

Nicoll, Wm. 1914.—The Trematode parasites of North Queensland. II. Parasites of Birds. Parasit., 7: 105-126.

Stossich, M. 1902.—Il Monostomum mutabile Zeder e le sue forme affini. Boll. soc. Adriat. sci. nat. Trieste, 21: 1-40.

ON SOME PROTOZOA PARASITIC IN FRESH-WATER FISHES OF NEW YORK*

R. Kudo

The Protozoa upon which the present communication is based, were found parasitic in fresh-water fishes collected in the vicinity of New York City, during August, 1920. Although no extensive research could be undertaken due to the small number of fishes obtained, yet new species of Myxosporidia as well as new host species were noticed. Furthermore, it became known that some of the common protozoan parasites of European fresh-water fishes of which little has been recorded up to date, occur also in North American waters. Therefore, I shall here briefly state my observations upon them.

Six species of fish were examined. *Abramis crysoleucus* (two individuals), *Ameiurus nebulosus* (four), *Anguilla chrysypa* (ten) and *Lepomis humilis* (three) showed no myxosporidian parasites. The blood of none of these fishes was examined so that I cannot make any statement regarding the blood-inhabiting Protozoa. The results of examination of *Lucius reticulatus* and *Moxostoma* sp. are as follows:

LUCIUS RETICULATUS

The host fish, thirteen in all, varied from 6.5 to 15 centimeters in length. On examination of internal organs, it was found that they were infected by three different protozoan parasites.

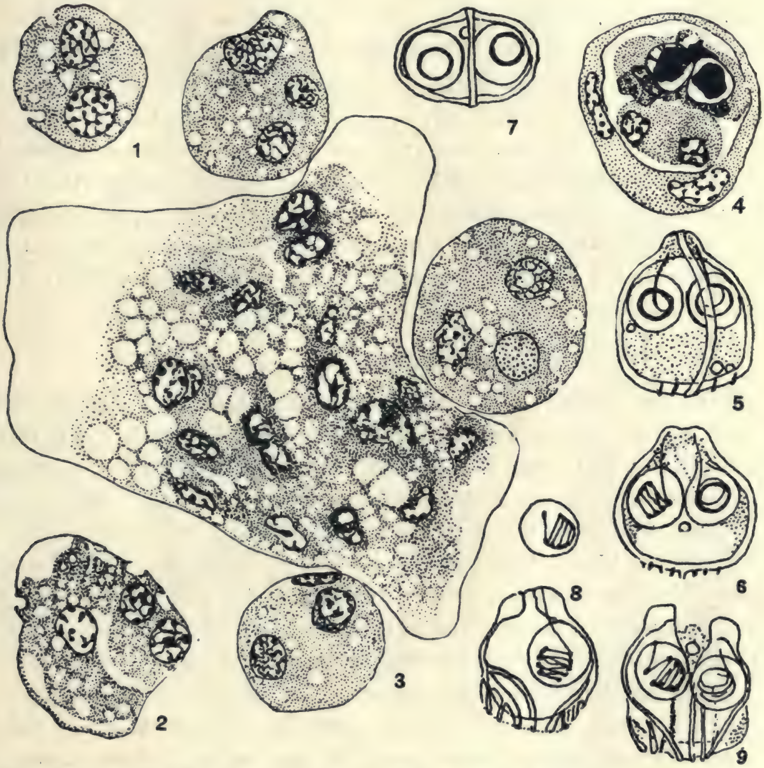
Wardia lucii nov. spec. (Figs. 1 to 9)

Habitat. In the uriniferous tubule, the space in the Malpighian body and connective tissue of the kidney. Two out of thirteen fish examined were found to be infected by the present Myxosporidian. In both cases the infection was light. A number of young vegetative forms and a few mature spores were observed in smears as well as section preparations.

Vegetative form. Form variable. It is usually rounded, viewed in fresh hanging drop preparations. The cytoplasm is distinctly differentiated into the homogeneous hyaline ectoplasm and highly vacuolated endoplasm (Fig. 3). Young forms in fixed preparations usually do not show the differentiation; the entire body is filled with granulated endoplasm (Figs. 1 and 2). Youngest forms are apparently uninucleate. The nucleus divides as the body becomes larger, producing a vegetative and a generative nucleus (Fig. 1); the latter divides soon afterwards into two (Figs. 2 and 3). Further changes seem to take

* Contributions from the Zoological Laboratory of the University of Illinois, No 184.

place in the ways similar to those of *Leptotheca ohlmacheri* which will be published elsewhere, including the formation of trinucleate gemmae which however are formed more than one in number in the present species (Fig. 3). Contrary to the above mentioned species, large forms (Fig. 3) which may produce a number of spores are also encountered, although no large cyst formation takes place. Lobose pseudopodia which vary in size according to the size of the trophozoite from which they are protruded, are slowly formed at unlocalized parts of the body. Polysporous and disporous.



Figs. 1 to 9, *Wardia lucii* nov. spec. Fig. 1, a binucleate trophozoite. Fig. 2, a trinucleate trophozoite. Fig. 3, a large trophozoite with three extruded gemmae (?). Fig. 4, a young spore. Fig. 5, the front view of a spore. Fig. 6, an optical section. Fig. 7, the end view of a spore. Figs. 8 and 9, spores slightly pressed under the cover glass. Figs. 1 to 4, Schaudinn, Giemsa, smears; $\times$ 2100. Figs. 5 to 9, fresh spores in physiological solution; $\times$ 2350.

Spore. Studied in hanging drop preparations, the spores show the following characters: Rounded triangular with convex sides in front view; oval in end views; and oblong in profile. The shell valves are thickened at the anterior tip. The sutural ridge prominent, is at right or acute angles to the line connecting the two polar capsules. Each

shell valve has irregularly marked ridges on its posterior portion. Two polar capsules spherical and usually equal in one and the same spore, occupy the anterior portion of the spore. The polar filament, coiled 4 to 5 times, is distinctly visible, and shows the similar condition as was shown by me (Kudo, 1920: 86, Figs. 620 and 621) in the case of *Mitraspora elongata*. The sporoplasm is homogeneous or finely granulated, and seems to fill the posterior half of the spore. Dimensions of fresh spores: sutural diameter 8 to 9μ, breadth 8 to 8.5μ, thickness 5 to 6μ, diameter of polar capsules 2.5 to 3.5μ, length of extruded polar filament (by pressure or potassium hydrate solution) 50 to 70μ. When stained, the sporoplasm appears distinctly to be a single mass containing two nuclei as is the case in the great majority of Myxosporidia.

The above description leads one to place the Myxosporidian in the genus Wardia (Kudo, 1920: 56) which I established (Kudo, 1920: 82-83) for *Wardia ovinocua,* the ovarian parasite of *Lepomis humilis.* The spores of both species were studied and measured in the fresh state, and show marked difference in every respect. The vegetative forms and the modes of spore formation are also entirely different one from the other. Hence, I consider that the present form is an unrecorded species, and have named it *Wardia lucii* nov. spec.

Myxidium lieberkühni Bütschli (Figs. 10 to 18)

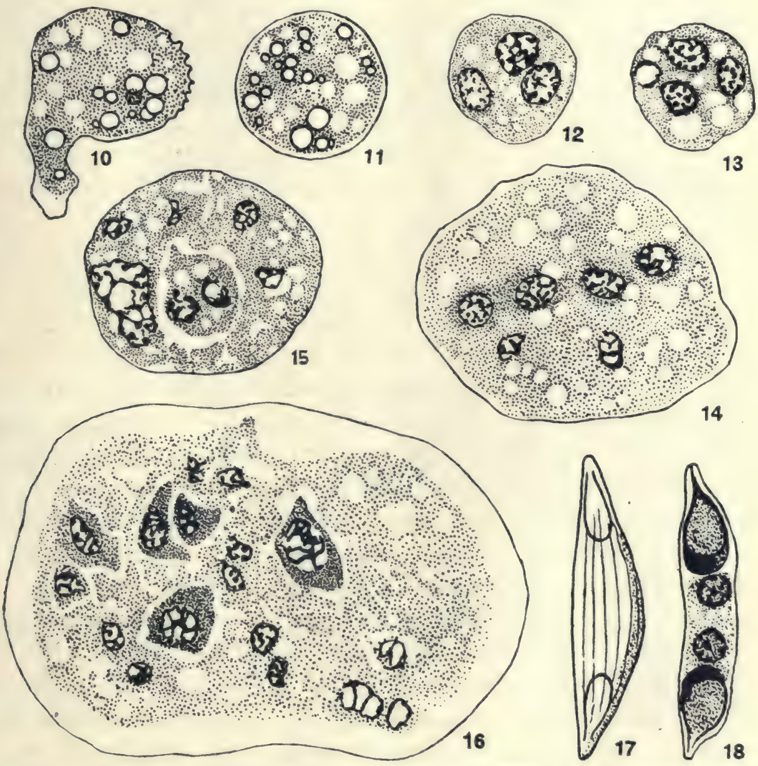
All the fish harbored the trophozoites and four also the spores of this well known Myxosporidian in their urinary bladders. *Myxidium lieberkühni* has been found by several European authors in the urinary bladder of *Lucius lucius* and *Lota lota* from various parts of Europe, and also by Mavor in the first named host species of Canada and the United States (Kudo, 1920: 107). Recently Debaisieux (1918) states that the Myxosporidian not only invades the bladder, but also the ureters, uriniferous tubules and further produces conspicuous cysts in the glomeruli of the Malpighian bodies of the kidney of *Lucius lucius.*

Lucius reticulatus is the third host species thus far found. Careful examination of the sections of the kidneys of the four host fish failed to confirm Debaisieux's observation upon the infected *Lucius lucius.*

Smaller fish showed apparently comparatively heavier infection than larger ones. Two fish, 6.5 and 6.8 centimeters long respectively, contained a large number of trophozoites and mature spores in their bladders. On the other hand, two large fish, 13.5 and 15 centimeters long respectively, showed a number of trophozoites and a few spores. The convincing evidence to explain this circumstance is missing. But the difference in the size of urinary bladders among small and large host fishes may hold some relation between the size of host fish and the sporulation of the Myxosporidian. The lack of space along the lining epithelium of the bladder in smaller fish, to which the tropho-

zoites attach themselves, may cause the sporulation in much shorter time than in larger fish where more space is found than the former.

Vegetative form. In every case, the trophozoites were small, the largest not exceeding 40μ in largest diameter. Form rounded or amoeboid (Figs. 10 and 11). Pseudopodia lobose or bristle-like (Fig. 10). Some individuals do not show any pseudopodium at all, examined even soon after the urine was made into hanging drop preparations (Fig. 11). The cytoplasm is distinctly differentiated into ectoplasm



Figs. 10 to 18, *Myxidium lieberkühni* Bütschli. Figs. 10 and 11, trophozoites viewed in hanging drop preparations. Figs. 12 to 16, trophozoites at various stages of development. Smear, Schaudinn, Giemsa, cedar oil; Fig. 16, the trophozoite is extremely thinly spread out. Fig. 17, a fresh spore. Fig. 18, a stained spore. Schaudinn, Giemsa. Figs. 10 and 11, $\times 1500$; Figs. 12 to 18, $\times 2100$.

and endoplasm (Figs. 10 and 11). The endoplasm is highly vacuolated and granular in structure, and contains droplets of oil. In contrast with the larger forms observed by European investigators, the trophozoites present in my preparations were strikingly uniformly small. I had an opportunity of comparing my smears and sections with a beautiful section preparation of the infected bladder of *Lucius lucius* pre-

pared by Professor A. Prenant of the University of Paris. Besides containing small trophozoites similar to those found in my preparations, the section showed numerous large individuals reaching 130μ in largest diameter. In the section preparations of the infected bladder of *Lucius reticulatus,* the small trophozoites are always seen attached to the epithelium, particularly, at the deepest part of the folds of the bladder. The changes that take place during the development of the trophozoites could not be studied in the fresh condition due to the pressure of other work conducted at that time. Uninucleate, binucleate and trinucleate trophozoites, particularly the latter, were found in a large number in stained preparations. In the trinucleate form, one can easily distinguish one vegetative and two generative nuclei, although the condition is in some individuals apparently reversed. Contrary to *Leptotheca ohlmacheri,* the vegetative nucleus divides repeatedly as the generative nuclei multiply. There is further seen that the division of the vegetative nucleus is far more active than that of generative nucleus. Consequently, as previous authors noticed, the trophozoites contain numerous nuclei of two kinds, one large and the other small. The distinction between the two kinds of nuclei is easily done in Giemsa stained smears, especially in very thinly spread smears, where one sees larger nuclei in dense islands of cytoplasm which stain in beautiful blue color, and the smaller nuclei simply scattered in the vacuolated endoplasm (Fig. 16). Some of the vegetative nuclei seem to undergo degeneration when the spore formation takes place. Viewed from the large number of uniformly small parasites, there most probably occurs active multiplication of the vegetative forms. Since I have not studied the fresh material thoroughly, I am unable to make any definite statement. In the stained preparations, I have not seen any indication that gemmation may take place in the present form as in *Leptotheca ohlmacheri.* On the other hand, I am inclined to think that plasmotomy most probably occurs. In sections, many trophozoites are found to form groups. In smears, I have quite frequently seen many small individuals connected with a somewhat larger one, the number reaching up to eight. In such young attached forms, the nuclei of two kinds were recognized, varying from 3 to 12 in number. Two spores are formed in one pansporoblast. These spores remain attached to each other even after being fully matured. Polysporous and disporous.

Spore. Studied in fresh state, the spores show the following characters: Form exactly as was figured by Bütschli (Kudo, 1920, Fig. 238). Fusiform bent to one side (Fig. 17). The spore membrane is comparatively thin. Sutural ridge hardly recognizable. The spore membrane is longitudinally striated. Polar capsules are pyriform and equal in one and the same spore. Contrary to the observations of

Thélohan and Mavor, the coiled polar filament is only faintly visible. Dimensions of fresh spores: length 18 to 20μ, breadth 4.5 to 5.5μ, polar capsules 5.5μ by 2μ.

When stained, the nuclei of the sporoplasm are seen rather widely separated from each other.

Trypanosoma remaki Laveran et Mesnil (Figs. 19 to 21)

Three fish were found to harbor in the blood a species of Trypanosoma, whose characters agree with those of *Trypanosoma remaki* described by Laveran and Mesnil (1902) and Minchin (1909). According to Laveran and Mesnil, the flagellate seems to have been observed by several authors in the blood of *Lucius lucius* from various

19 21 20

Figs. 19 to 21, *Trypanosoma remaki* Laveran et Mesnil. Figs. 19 and 20, var. *magna*. Schaudinn, Giemsa, cedar oil. Fig. 21, var. *parva*. Schaudinn, Heidenhain's iron hematoxylin. All $\times$ 3300.

parts of Europe. I have failed to find any North American record regarding this Protozoan.

According to the above mentioned three European authors, the number of the flagellates found in blood of the host fish is usually small. In the case of *Lucius reticulatus*, this was also true. The blood smears of two host fish showed one trypanosome in about every tenth field under the combination of compensation ocular 12 and apochromatic objective 16 mm., while that of the third host, one or two in every field under the same combination. In fresh as well as fixed and stained smears, active animals were only recognized. Viewed in hanging drop preparations, the flagellate undergoes very active movements by means of its undulating membrane and flagellum.

As were noted by the three previous investigators, two types of the animals which differ mainly in the dimensions of the body were also recognized in my preparations, although the distinction is not so sharply marked as Minchin stated (1909: 22). The larger type, var. *magna* (Figs. 19 and 20), measured after smears, fixed in Schaudinn, stained with Giemsa and mounted in cedar oil, varied from 30 to 33μ in length excluding the flagellum, and showed an average width of 2μ. The length of the flagellum seemed to vary, but could not be measured accurately due to its poor staining reaction.

The smaller type, var. *parva* (Fig. 21), measured in similarly prepared smears as above, showed the following dimensions: length of the body 24 to 27μ, average breadth 1.5μ.

The difference in the nature of the cytoplasm in the two subspecies observed by previous authors, was also noted. I have, however, not noticed in my preparations the "coarse granules staining reddish with Giemsa stain," observed by Minchin (1909: 22). Although not much difference in the size of blepharoplasts of two subspecies was noticed, they appeared larger in size than those figured by Laveran and Mesnil. The nucleus is usually located close to the anterior extremity. It is rounded oblong. In smears stained with Heidenhain's iron hematoxylin, a centrally located karyosome was surrounded by chromatic granules which were attached closely to the nuclear membrane. In Giemsa stained specimens, I have frequently noticed that the nucleus has two deeply staining masses located at opposite ends, the central part being apparently filled with a fluid which stained poorly (Figs. 19 and 20). Whether this is a stage in the division of the nucleus, I cannot decide at present. I have not seen any individual with two nuclei or two blepharoplasts so that I have no datum concerning the multiplication of the flagellate.

PARASITES FOUND IN MOXOSTOMA SP.

Myxidium moxostomatis nov. spec.

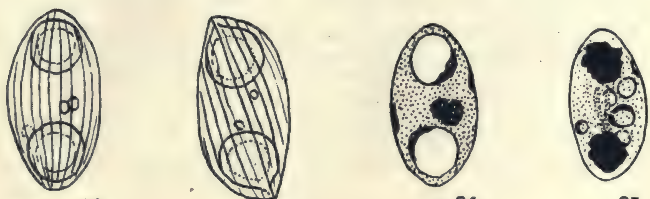
(Figs. 22 to 25)

Habitat. In the gall bladder of *Moxostoma* sp. Two fish were examined. One female, 30 centimeters long, showed a light infection by the present parasite, while the other male, 34 centimeters long, was found uninfected. Only a few spores and trophozoites were seen floating in the bile when examined.

Vegetative form. Large trophozoite is a rounded leaf-like body, the margin being frequently folded up in the bile. The cytoplasm is distinctly differentiated into ectoplasm and endoplasm. Further cytological study could not be carried out. No active amoeboid movements

of the body were noticed. The largest trophozoite observed reached a size of 2 by 1.5 millimeters. Each pansporoblast produces two spores. Polysporous.

Spore. Studied in fresh conditions, the spores show the following characters. Broad fusiform. Both ends are equally rounded in view at right angles to the sutural line; while they are pointed toward the diagonally opposite directions seen from a point on the sutural plane (Fig. 23). The spore membrane is comparatively thin. The sutural plane makes an acute angle with the longitudinal axis of the spore. The sutural line is straight, but is faintly marked. Fine striae, about ten in number on each shell-valve, run longitudinally. The polar capsules are spherical, and each is slightly drawn out toward its foramen. Coiled polar filament is invisible. Dimensions of fresh spores: length 8.5 to 10.5μ, breadth and thickness 5 to 6μ, diameter of polar capsules 3μ, length of polar filament 30 to 35μ.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Figs. 22 to 25, *Myxidium moxostomatis* nov. spec. Figs. 22 and 23, different views of fresh spores. Figs. 24 and 25, Giemsa stained spores. All $\times$2350.

When stained the sporoplasm shows occasionally a single but usually two nuclei. Dimensions of stained spores (smears): length 8 to 9μ, breadth and thickness 4.5 to 5μ, diameter of polar capsule 2.5 to 3μ.

From the above description, it is clear that the Myxosporidian is a species of the genus Myxidium. Compared with 27 species of the genus (Kudo, 1920), one finds that the dimensions of the spore do not agree with any one of them. In the shape of the spore, it is somewhat similar to *Myxidium macrocapsulare* (Kudo, 1920: 113), but is different in dimensions. Further comparison cannot be made, as the vegetative form of the latter species is unknown. Hence, I believe this Myxosporidian is a new species, and name it *Myxidium moxostomatis* nov. spec.

2 *Myxobolus* (?) sp.

In the smears of the kidney of a small fish, 9.5 centimeters long, a few isolated spores apparently belonging to the genus Myxobolus, were noticed. As the preparations were misplaced, detailed study could not be made.

In any case, no pathological changes in the external characters or internal organs, were recognized.

SUMMARY

1. Two new species of Myxosporidia, *Wardia lucii* and *Myxidium moxostomatis,* parasite in *Lucius reticulatus* and *Moxostoma* sp. respectively, of New York, are described.

2. A new host fish for *Myxidium lieberkühni* Bütschli is observed.

3. *Trypanosoma remaki* Laveran et Mesnil occurring in the blood of *Lucius reticulatus,* is studied.

Works Cited

Debaisieux, P. 1918.—Notes sur le *Myxidium lieberkühni* Bütschli. La Cellule, 30: 281-290, 1 pl.

Kudo, R. 1920.—Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Ill. Biol. Monogr., 5: 245-503, 25 pl. and 2 textfig.

Laveran, A., and Mesnil, F. 1902.—Des trypanosomes des poissons. Arch. Protist., 1: 475-498, 15 textfig.

Minchin, E. A. 1909.—Observations on the Flagellates, Parasitic in the Blood of Fresh-Water Fishes. Proc. Zool. Soc. London for 1909, pp. 2-31, 5 pl.

NOTES ON GREGARINES *

MINNIE WATSON KAMM

This paper includes the description of a single new species of gregarine, of the genus Gregarina, taken from a coleopteran host and notes on two species discovered by Leidy around the year 1850, belonging to the genus Stenophora and found in myriapods.

STENOPHORA LARVATA (Leidy) Ellis (Fig. 1, A and B)

Host: *Spirobolus spinigerus* Wood [*Spirobolus marginatus* (Say); *Julus marginatus* Say].
Location: Urbana, Ill., August, 1920.
Habitat: Intestine.

The present species was found by Leidy in 1849, and is interesting because it was the first of the long list of gregarines which he observed. He gave to it the name *Gregarina larvata* for "*Gregarina* is probably the larva condition of some more perfect animal, but . . . I have not been able to detect any form which could be derivable from it. . . . In the state in which *Gregarina* is found, it would probably hold a rank between the *Trematoda* and *Trichina*, the lowest of the Nematoidea."

The species has received many other names, being discussed by numerous subsequent workers. Labbé (1899) placed it in his newly-created genus Stenophora and Ellis (1913) returned to it the original species name. A detailed discussion of the species with references to the literature will be found in Watson (1916: 49-51). In this paper I stated "*Stenophora larvata* has not been found since Leidy's discovery of the species and its validity must be questioned until his work is substantiated by rediscovery of this parasite."

The present notes, then, reestablish the existence of the species, although they are confined to the mature vegetative stage only. I have opened numerous millipeds in different years but only in one instance succeeded in finding this parasite and then but few individuals were present. The longest specimen reached 550μ with a maximum width of 50μ and ratios of Length protomerite: Total length :: 1 : 22 and Width protomerite : Width deutomerite :: 1 : 2.5; Leidy's record stands Maximum length 800μ, maximum width 23μ; ratio LP : TL :: 1 : 20; WP : WD :: 1 : 2.

The protomerite is comparatively small, broadly dome-shaped, almost flat on top, and with a minute papilla at the apex. It is but slightly constricted at the septum where the outer layer (epicyte) is

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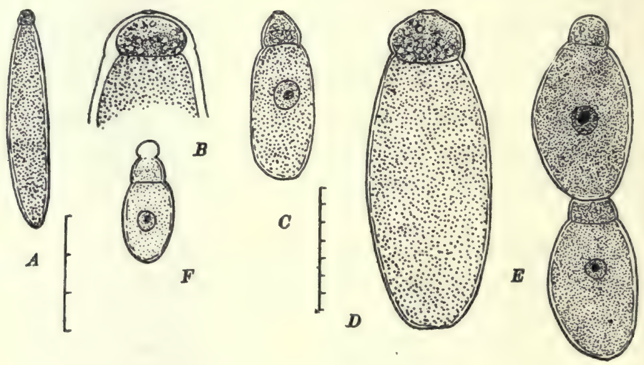
* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 185.

considerably thickened. Protoplasmic granules in the protomerite are much coarser, more sparsely scattered, and more refringent than those in the deutomerite.

The deutomerite is very large in comparison with the protomerite, elongate-cylindrical and broadly rounded posteriorly. It possesses considerable motility, a characteristic possessed by many of the Stenophoridae. The protomerite, however, is rigid. The endocyte is very dense and homogeneous, and is black in transmitted light. The nucleus is almost completely obscured in life, and is small and spherical.

The species, unlike many members of the genus, is very susceptible to a water medium, ceasing movements almost immediately. The protoplasm is collected in masses within five minutes although the epicyte does not rupture, practically the original shape being retained after half an hour on the slide. Many species in the genus remain alive and



EXPLANATION OF FIGURE 1

A, B, Stenophora larvata (Leidy) Ellis.

C, D, Stenophora polydesmi (Lankester) Watson.

E, F, Gregarina anthici nov. spec. (*this paper*).

The line under A represents 0.3 mm.; that under C 0.07 mm., the same magnification being used for B, C, D, E and F.

motile for this length of time. No cysts or sporozoites were found. One-half the infected intestine was reserved for sectioning, but no further evidence of parasitism was found.

A few representative measurements in microns are appended here:

	a	b	c
Length protomerite	40	40	20
Length deutomerite	510	490	420
Total length sporont.............	550	530	440
Width protomerite	50	60	50
Width deutomerite	110	130	110
Ratio LP:TL	1:13.7	1:13.3	1:22
Ratio WP:WD	1:2.5	1:2.1	1:2.5

STENOPHORA POLYDESMI (Lankester) Watson (Fig. 1, *C*, *D*)

Host: *Fontaria virginiensis* (Drury) [*Polydesmus virginiensis* Drury].
Location: Urbana, Ill., August, 1920.
Habitat: Intestine.

This species also was discovered by Leidy (1853), and its identity established by him. It has since been found and described by Crawley, in 1903. Some confusion concerning its nomenclature has arisen, and a discussion with synonyms will be found in Watson (1916: 51-2). It only remains to add a new habitat, a table of dimensions including those of the cyst, and minor characteristics of the vegetative stage.

The specimens found are shorter than those recorded by both Leidy and Crawley (900μ and 400μ respectively) but are undoubtedly not fully mature. Cysts were found measuring 600μ in diameter, which indicates much larger sporonts than any of the numerous ones I saw.

The protomerite is coarsely granular, with a dozen or more large angular transparent granules resembling grains of sand collected near the septum. There is a slight papilla at the apex with a grouping of protoplasmic granules to resemble a pore leading back into the protomerite, and which has been so frequently observed by Léger for this genus. I am unable to explain the latter phenomenon. The papilla is probably a rudimentary structure, possessed by most members of this genus.

The endoplasm of the deutomerite is not dense as in many of the species of the genus, and Brownian movement can be seen readily along the periphery. The nucleus is clearly visible *in vivo* and is small and spherical with one karyosome. One of Leidy's figures represents an ellipsoidal nucleus but at a place where contraction of the deutomerite is evident; his other figures represent it as spherical.

Measurements in microns of a few specimens follow:

	a	b	c	d
Length protomerite	30	30	20	20
Length deutomerite	150	145	80	70
Total length sporont......	180	175	100	90
Width protomerite	40	40	20	27
Width deutomerite	80	82	40	50
Ratio LP:TL	1:6	1:5.8	1:5	1:4.5
Ratio WP:WD	1:2	1:2	1:2	1:1.9
Diameter cyst	600	600	600	

GREGARINA ANTHICI nov. spec. (Fig. 1, E and F)

Host: *Anthicus* sp. fam. Anthicidae. (Det. Messrs. Malloch and Alexander.)
Location: Urbana, Ill., June, 1920.
Habitat: Intestine.

The host of this parasite is a minute beetle which frequently flew into my study through the meshes of the screen. Several hundred parasites were often found in an intestine, many of them associated in pairs. No anomalies were seen.

The body is obese, the deutomerite egg-shaped, widest at or just anterior to the middle and well-rounded posteriorly. The protomerite varies from a rather high to a somewhat flattened dome. There is no constriction at the septum in adults.

The protomerite is almost transparent, filled with coarse granules, while the deutomerite is homogeneous and finely granular, in adult specimens very dense and black except at the edges. The nucleus is faintly visible in life. In young specimens the deutomerite is tan-colored and not dense. The nucleus is spherical and contains one large karyosome.

Live trophozoites were seen through the intestine walls and were attached by means of a large simple knob typical of the genus Gregarina. Similar epimerited specimens were found free in the intestine. No cysts were found.

A few measurements of typical specimens in microns follow:

	Primite			Satellite	
	a	b	c	a	b
Length protomerite	20	15	20	19	15
Length deutomerite	90	75	80	79	55
Width protomerite	23	30	30	31	22
Width deutomerite	67	60	60	59	35
Total length sporont	110	90	100	98	70
Total length association	208	160			
Ratio LP:TL	1:5.5	1:6	1:5	1:5.1	1:4.6
Ratio WP:WD	1:2.8	1:2	1:2	1:1.9	1:1.5
Diameter nucleus	18	20		17	

Literature Cited

Watson, M. E. 1916.—Studies on Gregarines, Ill. Biol. Monogr., 2:211-468. 15 pl.

NOTES ON THE OCCURRENCE OF MONILIFORMIS SP. IN RATS IN TEXAS

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Rice Institute

During the summer of 1920, following the discovery of bubonic plague in a number of Gulf Coast cities, including Galveston and Beaumont, the writer was engaged in an examination of rats caught in the city of Houston, Texas, to determine the presence or absence of plague in that city.

The rat population of Houston is different from that of any other American city, as far as known to the writer, in that from 35 to 40 per cent. of the city rats belong to the species *Epimys alexandrinus,* the roof rat. In other American cities, including the neighboring cities of Galveston, Beaumont and New Orleans, the roof rat constitutes less than 5 per cent. of the total rat population. The wharf or Norwegian rat, *Epimys norvegicus,* constitutes about 60 per cent. of the total number in Houston, whereas the true black rat, *Epimys rattus,* is rare, probably less than 1 per cent. of the total. This rat is commonly confused with melanistic examples of the wharf rat, which are rather common, so that the statistics usually give a higher percentage of *Epimys rattus* than is actually true. A considerable number of rats, probably between 1 and 2 per cent., though resembling roof rats in their graceful form, showed the coloration of wharf rats, and had a tail and ears which were intermediate between the two species. Whether these rats should be looked upon as a distinct species, or, as seems more probable, as intergrades between *norwegicus* and *alexandrinus,* has not been determined.

The most interesting parasitological fact brought out by the examination of these rats is the common occurrence of Acanthocephala of the genus Moniliformis (Travassos 1915). Up to the present time these worms have been reported from the United States only in three instances. Ward in 1917 described these worms from a squirrel in Illinois. He considered his specimens as belonging to a species distinct from the Old World form and named it *Hormorhynchus* (=*Moniliformis*) *clarki;* a full description of this form has not yet been published. Worms of the same genus were previously reported by H. C. Chapman (1874) and by Stiles and Hassall (1984) from *Sciurus vulpinus* and *Sciurus niger,* respectively. Dr. Van Cleave has informed the writer that he has specimens of Moniliformis from Oklahoma also.

Although the full statistics are not yet available, the percentage of adult Houston rats which are infected is approximately 10%, the percentage of infected *E. alexandrinus* being higher than that of

E. norvegicus. The number of worms found in a single rat varies from two or three up to between one and two hundred. Not infrequently the entire small intestine from just behind the stomach to the ileocecal valve is crammed full of the worms, and distended to several times its normal diameter. The intestinal walls become thin and flabby from the distention, and the circumference of the small intestine may exceed three centimeters. With a few exceptions the female worms which are mature, i. e., bear eggs with developed embryos, vary from a minimum

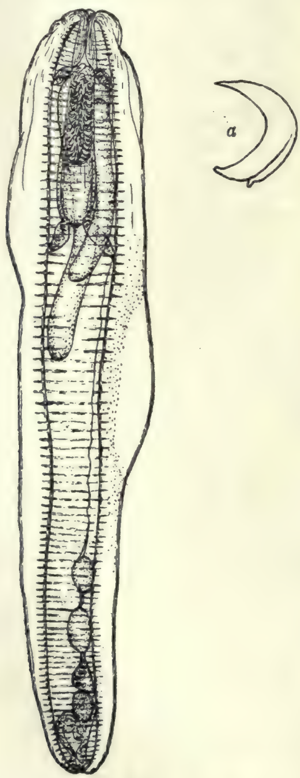

Fig. 1.—Immature acanthocephalan from Norway rat, *Epimys norvegicus,* showing retracted proboscis and immature male reproductive organs. × 30. *a,* Hook from proboscis of same worm. × 385.

of 140 mm. to a maximum of 270 mm. in length. The body is very heavily annulated throughout most of its length. The annulations begin very fine, a few millimeters behind the cephalic extremity, become coarse over the anterior two-thirds of the body, and then become gradually less pronounced, the terminal ten to twenty millimeters being practically smooth. The diameter of the enlarged portions of the pseudo-segments reaches a maximum of 3.2 mm., the adjoining constricted portion being from 2 mm. to 2.5 mm. in diameter. When freshly extracted from the

intestine the worms are flabby and flattened and resemble taeniae. The proboscis measures from 0.55 mm. to 0.6 mm. in length, with a maximum diameter of 0.15 mm., about one-fourth the distance from tip to base. The hooks are irregularly arranged; their quincunxial disposition is not as apparent as in many Acanthocephala. There are in all from 17 to 20 hooks in a longitudinal series, if the more or less alternating ones in two adjacent rows be counted. There are six hooks in a transverse row, counting only those in alternate longitudinal rows. The lemnisci, even in the largest specimens, do not exceed 5 mm. in length, and are usually little over 4 mm. The male worms measure from 30 to 45 mm. in length, the annulations being close together and much less conspicuous than in the female. The testes are less than 2 mm. in length and either touch each other or are separated from each other by a very short interval. The prostate glands are crowded together into an oval mass about 1.25 mm. long; the individual glands are almost indistinguishable. The eggs measure from 112 to 120μ by 56 to 60μ, thus conforming more closely with the measurements given by Travassos in Brazil (124 to 127μ by 71 to 74μ) than with those usually given by European writers. The embryos inside the eggs measure from 80 to 84μ in length.

In two or three Norway rats specimens of Moniliformis were found which were uniformly smaller than the worms usually seen. In these rats egg-bearing females measured from 55 to 90 mm. in length. One Norway rat contains numbers of these small mature female worms, measuring from 55 to 70 mm. in length and less than one mm. in diameter, and other larger specimens measuring from 90 to 270 mm. in length of which only those females which measure 150 mm. or more bear eggs. Some of the males from this rat measure only about 25 mm. in length. That the small worms, the dimensions of egg-bearing specimens of which average less than one-third that of the large egg-bearing specimens, especially when occurring in the same host, represent a single species seems to the writer highly improbable. Dr. Van Cleave suggests that the difference may be explained on the basis of periodicity, the smaller worms representing a more recent infection. This, however, does not explain the fact that the smaller representatives of the large worms, up to nearly three times the length of the smallest egg-bearing specimens, are immature, if lack of development of eggs can be accepted as a sign of immaturity.

Without specimens from other places for comparison, it is impossible to draw conclusions as to the status of the species of Moniliformis, three of which have been described up to the present time. The type species, *M. moniliformis* Bremser is described very differently by different authors, and it is possible, as suggested by Lühe (1911), that as usually accepted it is a composite of more than one species. The specimens described by various writers from rats in Southern Europe, mea-

suring from 6 to 8 or 10 cm. in length, may not be identical with the specimens from Arvicola and Cricetus, measuring from 12 to 27 cm. in length. What the relation of either of these is to the forms found in Houston, or to those described by Ward from a squirrel in Illinois as *M. clarki.* or to the South American forms from rats described by Travassos, or to *M. cestodiformis* described by von Linstow from a hedgehog in West Africa, can only be determined by careful comparison of specimens from all these places and hosts. Inasmuch as his work has already been undertaken by Dr. Van Cleave at the University of Illinois, who is collecting material from various parts of the world, the specimens from Texas will be submitted to him for comparison with other forms.

In a single specimen of *Epimys norvegicus* twelve specimens of a small Acanthocephalan worm were found in the lower portion of the small intestine. These worms were at first thought to constitute an entirely new species, but more careful examination makes it appear probable that they are immature specimens of Moniliformis. The specimens are all of approximately the same size, and could very possibly have all been acquired from the ingestion of a single intermediate host. None of the females show any signs of developing eggs, and the males, though showing all of the male reproductive organs present, possess them in an evidently immature state. The worms are flattened and show no signs of external annulation; they taper gradually from just back of the proboscis sheath to the posterior end. Their length is from 3.2 mm. to 4 mm., the greatest width about 0.5 mm. The proboscis is retracted in all but one of the specimens, thus making it difficult to count the hooks, but there are evidently about 14 in a longitudinal row, and approximately six or seven in a transverse series. The hooks are all practically alike in size and form. Their characteristic shape is shown in figure 1*a*. The proboscis sheath is relatively large and measures about 0.75 mm. in length; it is retracted some distance within the anerior end of the body. There is a pair of powerful and conspicuous retractor muscles attached to its posterior end and a second pair at its anterior end. The lemnisci are relatively large, about twice as long as the proboscis sheath. The condition and arrangement of the male reproductive organs is shown in figure 1. The ladder-like lacunar system is very conspicuous, as shown in the figure.

Until further information can be obtained it is impossible to say whether these young worms represent a Moniliformis or whether they represent an entirely new genus, but the presumption is strongly in favor of their being Moniliformis.

A number of infection experiments with both the large and small forms of adult Moniliformis were carried out on cockroaches of two species, namely, *Periplaneta americana* and *Blatella germanica,* by feeding both adult and young roaches on the feces of infected rats and on the egg-filled bodies of the worms, but without any successful results in any case. The fact that a rather high percent of rats from rice and grain mills and storage houses were infested seems to indicate that some grain insect, possibly the rice beetle, *Calandra oryzae,* may act as intermediate host. It is hoped that this point may be cleared up in the near future.

Literature Cited

Chapman, H. C. 1874.—On Echinorhynchus Moniliformis. Proc. Acad. Nat. Sci. Phila., pp. 76.

Linstow, O. von. 1904.—Neue Helminthen aus Westafrika. Centralbl. Bakt. Paras., I Orig., 36: 379-383, 1 pl.

Lühe, M. 1911.—Acanthocephalen Süsswasserfauna Deutschlands, Heft 16.

Stiles, C. W., and Hassall, A. 1894.—A Preliminary Catalogue of the Parasites Contained in the Collections of the U. S. Bur. An. Ind., U. S. Army Med. Mus., Biol. Dept. U. Penn. (Coll. Leidy) and in Coll. Stiles and Coll. Hassall. Vet. Mag., 1: 245-253, 331-354.

Travassos, L. 1915.—Revisão dos acantocefalos brazileiros. I Fam. Gigantorhynchidae Hamann 1892. Brazil-Medico, 29, no. 12, p. 89.

1917.—Contribuiçoes para o conhecimento da fauna helmintologica brazileira. VI. Revisão dos acantocefalos brazileiros, Parte I, Fam. Gigantorhynchidae Hamann 1892.

Ward, H. B. 1917.—*"Echinorhynchus moniliformis"* in North America. Jour. Parasit., 3: 141, 182.

A CASE OF URETHRAL MYIASIS

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In 1898 I had the chance to observe for the first time in Roumania cases of myiasis. These observations were published in the Archives de Parasitologie and also another case in 1900. This time of the four larvae abstracted from an abcess in the gum one transformed into a fly (*Sarcophaga wohlfahrti*). I communicated other cases in 1912 and again in 1913 a case of creeping disease to the Centralblatt für Bakteriologie.

In July, 1920, a student, M. N. T., 22 years old, came to me and related that before going to bed he had urinated eleven worms which he brought in alcohol. Some days before he had felt at intervals slight tickling sensations accompanied by erections and sometimes by ejaculations. On examination of the specimens under the microscope we recognized normal larvae of *Musca domestica,* about 6 mm. long.

Certain authors, among them Leuckart, have declared that it was impossible for insect larvae to live in the urethra or bladder on account of lack of air. However we believe with R. Chevrel that they find in these organs the biological conditions indispensable to their existence and development; obscurity, humidity, heat, nourishment and oxygen. Nourishment is supplied principally by the mucopurulent secretion, or the albuminoid filtrate which lines the walls of the bladder or urethra. They obtain oxygen either from the outer air or from the gas which the bladder contains in a free state.

I did not think that this case could be a fake, for the young man was a very serious student, robust and in good health. I expressed doubt about the cleanliness of the vessel, but the student assured me that the urine was clear and the smallest foreign body could be seen even at the bottom of the white vessel. I then examined the organ to see if by chance flies might not have deposited eggs under the prepuce where the larvae might have made their way into the urethra. The organ was in a state of perfect cleanliness so I had to abandon this hypothesis.

I recalled the case of Edouard d'Haeneus (1898); a patient who had eliminated from the urethra larvae of the domestic fly had the habit when bathing to inject water into the urethral canal. In this case he probably injected into the bladder with the water fly eggs and these were later transformed into larvae. The young man said that he had never injected water, that formerly when attacked by blennorhagia

he had made medicinal injections, but that after his cure (three months previously) he had not made a single further injection. In order to be convinced that there was really no trace of discharge, I told him to press the organ tightly at the base. As he did this he declared that all at once he felt a tickling sensation. The organ immediately became erect while the patient showed pain and evacuated in my presence from the urethra with the sperm eight fly larvae resembling in every respect those which he had brought the day before in alcohol. The occurrence leaves no room for doubt that this is an authentic case of myiasis of the urinary passage.

July, 1920, was very warm at Jassy. The young man told me he slept at night without pajamas covered only with a sheet which he threw aside at times on account of the extreme heat. The slight discharge still present as the last trace of the blennorhagia had been sufficient to attract the flies, which as is known have a very well developed sense of smell. They had deposited their eggs in the neighborhood of the urinary meatus, and larvae had penetrated into the urethra and perhaps even into the bladder.

The explanation which R. Chevrel gives of the penetration of fly larvae agrees fully with this case. The genitalia, imperfectly protected by clothing, are visited in the early morning by flies attracted by the warmth of the body; if at this time menstruation, recent emission, or discharge from the bladder or urethra has left in the neighborhood of the urinary meatus any trace of organic matter the flies may easily deposit their eggs there. The victims, deep in sleep, cannot protect themselves. Placed under the edge of the prepuce or in the folds of the vulva the eggs so protected and maintained at a high temperature will hatch at the end of a few hours and endowed with remarkable vigor and activity the fly larvae incessantly creep about until they end by finding the urinary meatus. Thanks to their insignificant size they circulate in the mucus without provoking sensible tickling sensations and pass easily into the urethra.

I hoped that the larvae would be transformed into nymphs, but two days later all eight were dead. I saw the young man again a month later. He told me he had not felt a single one of the symptoms he had noted previously. Probably all the larvae had been eliminated naturally.

References Cited

Chevrel, R. 1909.—Sur la Myase des voies urinaires. Arch. Parasit., 12: 370-450.

Haenens, E. 1898.—Myiasis du canal de l'urèthre. Ann. Bull. Soc. méd. d'Anvers, 60: 101-112.

Léon, N. 1898.—Quelques cas de Myase observés on Roumanie et leur traitement par les paysans. Arch. Parasit., 1: 314-315.

1900.—Notes de parasitologie Roumaine. Arch. Parasit., 3: 228-236.

1912.—Notes de parasitologie. Centralbl. Bakt. Par., I. Orig., 78: 382.

1913.—Un cas de Creeping disease en Roumanie. Centralbl. Bakt. Par., I. Orig., 72: 384.

SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The forty-fifth meeting of the society was held September 17, 1920.

Dr. Stiles presented an informal report on the prevalence of entamebae in man in the United States, noting that infection with amebae was probably more prevalent than was generally believed and citing Kofoid's work in this connection.

Dr. Yoshida presented an abstract of a paper by Dr. Y. Saheki, on experiments resulting in the successful development of *Hymenolepis nana* in man, rats and mice by feeding eggs from the tapeworms of this species from man.

Mr. Wigdor presented a note on an unusual and a new species of Diphyllobothrium in an imported dog, in which he reported the collection of *D. fuscum* and of a new species of *Diphyllobothrium* from an imported Russian sheep dog. The new species resembles most closely *D. cordatum.*

Dr. Schwartz presented a new record of *Hymenolepis diminuta* from man in the United States. The record is based on a number of gravid segments collected from the stool of a child 2½ years old by the child's mother and sent to the laboratory by Dr. C. C. DuBois of Warsaw, Indiana, with the statement that the child had passed several feet of tapeworm on a previous occasion. Dr. Schwartz noted five published records of this parasite from man in the United States, reported by Weinland (1858), Leidy (1884), Packard (1900), Deaderick (1907), and Nickerson (1911). In discussion, Dr. Stiles reported three unpublished cases, making a total of nine cases, of this worm in man in the United States. Dr. Cort reported that Dr. Malloy had found a case of the same sort in Nicaragua.

Mr. Chapin reported the finding of *Hymenolepis farciminosa* in the starling, *Sturnus vulgaris,* in this country, indicating that this tapeworm parasite of the bird in Europe has been successfully established in this country.

Dr. Cobb reported that *Anguillula silesiae,* the eelworm occurring in the mats on which the Germans placed their beer steins, had now been found by him in rotten peaches. Apparently it feeds on an organism found in decaying peaches and probably on the same organism in beer mats. It apparently finds favorable conditions in a medium containing a certain content of acetic ether.

Dr. Hall presented a note regarding Cuterebra larvae from cats, with a list of those reported from other hosts. Two new cases from cats were presented, one being a case from Washington, D. C., where the larva was present in the neck, and one being a case from Nashville, Tenn., where the larva was collected from the nostrils. What appear to be similar cases of Cuterebra in the cat have been reported in at least eight instances previously. The paper included a summary of published and some unpublished cases of larval Cuterebra (including Bogeria and Rogenhofera, subsequently separated from Cuterebra) from various hosts, and a key to the genera Cuterebra, Bogeria and Rogenhofera.

Dr. Hegner gave an informal talk on European parasitologists and the conditions of their laboratories since the war, having just returned from a trip in which he attended the unveiling of the monument to Eduard van Beneden, who was, among other things, a parasitologist.

Dr. Cort presented a note on sex in the Schistosomes (since published in *Science,* 53:226-228).

The forty-sixth meeting of the society was held October 23, 1920. Dr. Hassall was elected president and Dr. Hall secretary.

Dr. Cort called attention to a species of fluke described from man in Japan by Onji and Nishio in 1915. This work was done in Miyairi's laboratory, but has apparently never been noted in the literature outside of Japan or in

any language except Japanese. The fluke, *Heterophyes nocens,* occurs in the southwestern part of the largest of the Japanese islands and was first found by Onji, a practicing physician. It is only known from two villages and occurs in 22 to 30 per cent. of their inhabitants. The final larval stage occurs in a fish, *Mugil japonicus,* which is customarily eaten raw. The life history has been experimentally demonstrated by feeding infested fish to dogs. In the primary host, the fluke occurs free in the intestine or attached to the intestinal villi. It is very similar to *Heterophyes heterophyes,* but is smaller. *H. nocens* is 0.9 to 1.1 mm. long. The rodlets of the genital sucker are about sixty in number, instead of seventy to eighty as in *H. heterophyes.* The intestinal ceca are unequal in length and extent. The encysted larva is one-third as long as the adult. When these larvae are fed to dogs, they become mature and egg production begins in seven to eight days.

Dr. Cort also noted that the eggs of *Metagonimus yokogawai* were long confused with those of the more dangerous *Clonorchis sinensis* and that the latter worm is not as common as was once thought. The eggs of Clonorchis are often shouldered at the operculum, whereas those of Metagonimus and Heterophyes are not shouldered, but have a smooth oval outline.

Dr. Bartsch called attention to the fact that the parasitologists had not been represented at the Pan-Pacific Congress, though the nature of the Congress was such that they should have been represented. He also noted the receipt from Dr. Yoshida of specimens of the snail which is the intermediate host of *Schistosoma japonicum* from Dr. Yoshida; the correct name of the snail is *Blanfordia japonica* (Adams, 1861).

Dr. Bartsch reported that a number of Japanese molluscs had been introduced into California and established there, and commented on the danger of such introduction, noting that Paragonimus had successfully established itself in Peru. This danger was emphasized in the discussion by Dr. Cort, and Dr. Stiles noted the occurrence of a doubtful case of schistosomiasis in the southern United States.

Dr. Boeck reported the examination of 157 individuals for amebae. A 1 per cent. aqueous solution of eosin was found to stain everything in a smear preparation of feces except protozoa and certain fungous cysts. An ordinary smear requires a half hour to examine thoroughly for protozoa, whereas with suitable technique it is expected to cut this time to ten to twelve minutes. At least two preparations are examined before a negative report is made. The iodine stain is used to differentiate species of amebae, and, if necessary, iron hematoxylin is used. Permanent preparations are made and filed. The examinations are complicated by the occurrence of flagellates. So far only two cases of *E. histolytica* have been found, with over twenty cases of *E. coli,* and about ten cases of Giardia. There were a total of fifty-seven cases of protozoan infections, eight cases where two species of parasites were present, and three cases where three species of protozoa were present, one of these cases being complicated by hookworm infestation. Dr. Ransom reported that he had once been infected with Chilomastix, the infection terminating of itself without treatment.

Dr. Ransom presented a note by Dr. Raffensperger on ascariasis in swine. Of ninety-three pigs farrowed from ten sows on a farm in Illinois, late in September, only 55 were left alive in October. Two of these were killed and examined postmortem, numerous ascarid larvae being found in the lungs. Seven larvae were found on one slide in a bit of mucus from the air passages, and twenty-two young worms were found in a small amount of the duodenal contents of one of the pigs. The pigs showed symptoms of "thumps." The previous year worm-infested pigs had been kept several months in a small orchard, where they had also passed numerous ascarids after anthelmintic treatment. This orchard, which had thus become badly contaminated, was used as a pasture for the sows and new-born pigs above mentioned. The findings in this case and similar cases speak for the abolition of the permanent hog lot so far, at least,

as young pigs are concerned. Clean pens, clean udders in the case of sows at the time of farrowing, and clean or only slightly contaminated pasture can be secured under farm conditions and will prevent serious infestation.

Dr. Ransom also reported the guinea-pig for the first time as a host of *Hymenolepis nana,* or *H. n. fraterna* if one regards the form from rats as a variation of the form in man. A guinea-pig had been kept with rats and became infested, presumably from the rats. It had about a dozen of these tapeworms, all immature, the largest being 3 mm. long and having fifty to sixty segments. The primordia of the genitalia were present.

Mr. A. H. Clark discussed the questions of parasitism, commensalism, etc. among marine animals.

Dr. Hegner presented a note in regard to bodies found in the red blood cells of the bull frog and the green frog on Long Island, New York (published in this number of the JOURNAL).

Dr. Stiles reported more fully on his three unpublished cases of *Hymenolepis diminuta* from man, briefly mentioned at the previous meeting of the society. In one case the specimens were collected by Dr. Talcott at Greenwood, Neb., in 1906; in another by Dr. Constance at Washington, D. C., in 1911, and in another by Dr. Leonard at Gastonia, N. C., in 1912.

Dr. Stiles also presented the following note:

Ascaris lumbricoides caught in the eye of a shoe-button. (Published in *J. Am. M. Assn.,* 76:239.)

The forty-seventh meeting of the society was held at Baltimore, Md., November 20, 1920.

Miss Cram presented the following note:

A CASE OF NANISM IN STRONGYLUS VULGARIS AND OBSERVATIONS ON THE LEAF CROWNS IN SPECIES OF STRONGYLUS

An abnormal specimen of *Strongylus vulgaris* was collected by Dr. Hall from a horse at Bethesda, Md. The specimen is a male of the same width as the normal males of this species, but is only about half as long, being 8 mm. long instead of 15 or 16 mm. In all other respects it appears normal.

Fig. 1

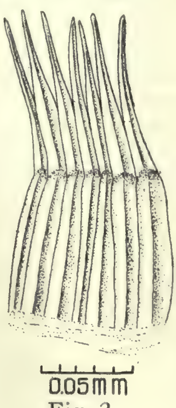

Fig. 2

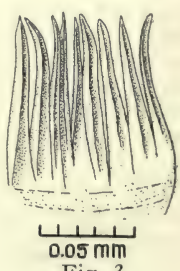

Fig. 3

When mounted the head was bent in such a way as to give a view of the anterior extremity as seen from the front and side, and it was noted that the outer elements of the leaf crown were not the simple structure ordinarily observed in strongyles possessing leaf crowns, but were split up into smaller elements (Fig. 1). On dissecting several normal specimens of *Str. vulgaris,* it was discovered that each of the initial basal elements was fringed out in the distal portion to form several finer tips, some elements showing as many as eight of these divisions. The elements of the leaf crowns of *Str. equinus* and

Str. edentatus, on the other hand, when similarly dissected, proved to be the usual simple, one-pointed structures. Those of *Str. edentatus* (Fig. 2) are slightly more complex than those of *Str. equinus* (Fig. 3), as the former have a thick ridge at about half the length of the leaves from the base, the distal portion bending forward and in toward the longitudinal axis of the worm, a feature which is not present in the quite simple leaf elements of *Str. equinus.* These variations from the extremely simple leaf elements of *Str. equinus* through the slightly more complex elements of *Str. edentatus* to the elaborate elements in *Str. vulgaris* do not seem to have been previously noted.

Dr. W. G. MacCallum presented the following note:

CHEMOTHERAPY IN INFESTATIONS WITH DIROFILARIA IMMITIS

Dogs imported into the Fiji Islands commonly die in the course of eighteen months as a result of the plugging of the pulmonary artery with *Dirofilaria immitis.* The writer undertook to investigate the effect of a number of drugs on the larvae in the blood by tests *in vitro* under the microscope. Most drugs were found to be ineffective in a dilution compatible with their administration by intravenous injection. Quinine was lethal to the parasites in dilutions of 1:5,000 and emetin in dilutions of 1:7,000. On intravenous injection in these dilutions, quinine killed the dogs before it killed the worms, but emetin was tolerated. The efficacy of the emetin treatment was not ascertained.

In the discussion Dr. Ransom noted Rogers' use of tartar emetic (the sodium salt) for filarial infestations, and Dr. Hall noted Schultz' use of collargol for filariae. It was also noted that the record of *D. immitis* from man is an error due to mislabeling. Dr. Grant reported that he had given tartar emetic in doses of 1 to 2 grains in 1 per cent. solutions without obtaining conclusive results.

Dr. Hegner reported the finding of a new blood parasite in the frog. The parasite is a protozoan which occurs free in the blood stream. It has the form of a disk with an undulating membrane originating on the concavity of the disk. The same frogs contain trypanosomes and Lankesterella in the blood, raising the question as to whether the new form is a stage in the life history of one of these forms.

Dr. Ransom presented a note by Drs. Ransom and Raffensperger on the development of *Arduenna strongylina* in the guinea-pig. Last summer Dr. Ransom examined speciments of *Onthophagus hecate* and other species of coprophagous beetles collected in hog pens near Bloomington, Ill., and found them commonly infested with larval nematodes, 1 to 2 mm. long, encapsuled in the body cavity. These larvae correspond to forms recorded by Seurát (1916) as occurring in coprophagous beetles in Algeria and identified by him as the larvae of *Physocephalus sexalatus* and *Arduenna strongylina,* the adults of which are common parasites in the stomach of the hog. The larvae of the two species as described by Seurat are very similar but may be distinguished from one another by the different location of the nerve ring and the presence or absence of spines on the tip of the tail. Recently Dr. Raffensperger at Chicago fed ten larval nematodes isolated from coprophagous beetles collected in a hog pen to a guinea-pig. Eleven days later he killed the guinea-pig and recovered from the contents of the pyloric portion of the stomach a small nematode which he suspected was an *Arduenna strongylina.* The specimen has been examined by Dr. Ransom and found to be a male of the species in question in the last larval stage preceding the adult stage, indications of an ecdysis about to occur being evident. It measured 5.8 mm. in length with a diameter of 160μ in the middle of the body. The full grown adult male commonly measures 10 to 15 mm. in length and 300μ or more in maximum diameter. Accordingly the young worm from the guinea-pig, had it been permitted to continue its development, besides undergoing an ecdysis which was impending, would have had to grow considerably before attaining the full size of the adult as it is commonly

found in hogs. During a period of 11 days in the guinea-pig it had grown to about three times the size of the larva as it is found encapsuled in beetles, and had undergone probably one molt, meanwhile developing from the third to the fourth or last larval stage and reaching a point in its development at which it was nearly ready to transform into the adult stage. Including the present case the nematodes recorded as occurring in the guinea-pig are *Trichinella spiralis, Gongylonema neoplasticum, Ascaris lumbricoides* and *A. suum* (larval stages), *Arduenna strongylina* (last larval stage), all from artificial infections with which may also be included various species of hookworms and Strongyloides whose larvae will live temporarily in this host, and finally *Paraspidodera uncinata* (natural infestation). The last named species was originally recorded by Travassos (1914) from the large intestine of the guinea-pig and related rodents in South America. It has been found by Ransom in guinea-pigs reared at Bethesda, Md., but apparently has not heretofore been reported in the United States.

Dr. Ransom also presented a note on some unusual parasites of the domestic hog. Three specimens of hookworms collected by Dr. S. Hadwen from the stomach of a pig at the Central Experimental Farm, Ottawa, Canada, September 4, 1919, were forwarded to the U. S. Bureau of Animal Industry for determination. These specimens, all females, two of which were immature, have been found to be *Uncinaria stenocephala* Railliet or *U. polaris* Looss. *U. stenocephala* occurs in dogs in Europe and *U. polaris* in foxes in North America. The two species are probably identical. In the Bureau of Animal Industry collections are some specimens of the common hookworm of sheep, *Bunostomum trigonocephalum,* labeled as collected from the pig by Dr. Cooper Curtice in May, 1890. In the same collections are also specimens of nematodes somewhat resembling hookworms collected from the small intestine of pigs by Dr. F. L. Kilborn at Washington, D. C., in May, 1890, and by Ransom at Bethesda, Md., in October, 1906. These belong to the species *Crassisoma urosubulatum* described by Alessandrini in 1909 from the pig in Italy. So far as known it has not heretofore been reported in the United States.

Dr. Schwartz presented a note on the Effect of Secretions of Certain Parasitic Nematodes on Coagulation of Blood. (Published in the JOURNAL, 7 : 144.)

Dr. Root presented the following note:

A CASE OF INTRA-UTERINE PUPATION IN THE SHEEP TICK

The sheep tick, contrary to the usual condition among insects, is pupiparous, and the egg is not deposited as such, but is carried in the body until it forms a puparium. In a number of specimens recently examined, one tick was found dead and on dissection was found to have in the uterus a puparium containing a fully mature young tick. In this case the death of the mother tick did not stop the development of the young tick, contrary to what is true of such flies as the tse-tse.

Dr. Simon presented the following note:

GIARDIA IN FIELD MICE IN NOVA SCOTIA

Of the field mice in Nova Scotia, 85 per cent. are affected with Giardia, apparently *G. microti* of Kofoid. The finding is of interest in connection with the question as to whether man becomes infested with Giardia from rodents. Grassi thought this was the case, believing that infection occurred through the feces. But rodents have been found to harbor several species of Giardia. *G. microti* closely resembles the human form. Attempts by the writer to infect wild rats with the human species have failed, though these rats could be infected with *G. muris* and the infection carried successively through white rats to white mice and vice versa. An experiment is now under way to determine whether the species from field mice in Nova Scotia can be transmitted to wild rats by feeding.

Dr. Boeck noted the presence of *Giardia microti* in *Peromyscus gambelli.* Dr. Boeck also presented the following note:

A PROTOZOAN SURVEY OF AN INDUSTRIAL SCHOOL FOR BOYS AND GIRLS

Total number of cases examined		83	
Total number of examinations		444	
Average number of examinations per case		5.3	
Total number of positive examinations	250		
Total number of negative examinations	194		
Total number of positive cases		66	79.5%
Total number of negative cases		17	20.4%
Number of persons infected with protozoa		64	77.1%
Number of persons infected only with protozoa		55	66.2%
Number of persons infected with worms		11	13.2%
Number of persons infected only with worms		2	2.4%
Number of persons infected with protozoa and worms		9	10.8%
Number of cases of:			
E. histolytica		9	10.8%
E. coli		41	49.3%
Endolimax nana		5	6.0%
Iodamoeba bütschlii		1	1.2%
Giardia intestinalis		40	48.1%
Chilomastix mesnili		1	1.2%
Hymenolepis nana		4	4.8%
Hookworm		2	2.4%
Ascaris		4	4.8%
Oxyuris		1	1.2%
Trichuris		2	2.4%
Cases with a single infection of protozoa		37	44.5%
Cases with a double infection of protozoa		22	26.5%
Cases E. coli and Endol. nana	2		
E. coli and E. histolytica	3		
E. coli and Giardia	14		
E. coli and Chilomastix	1		
E. histolytica and Giardia	1		
Giardia and Endol. nana	1		
Cases with triple infection of protozoa		4	
E. histolytica and giardia	2		
E. coli, E. nana and Giardia	2		
Quadruple protozoan infection		1	
E. histolytica, E. coli, Ioda., Giardia	1		
Single worm infections		10	
Triple worm infections		1	

In the discussion of Dr. Boeck's paper, Dr. Simon noted the theory that infection with these parasites was most apt to occur in the "crawling stage" of childhood, with its accompanying difficulty in the matter of proper sanitation. In this connection he noted that field mice could be infected with Giardia in the absence of water. Dr. Welch called attention to the fact that variations in the degree of protozoan and worm infestation reported by various observers was due in part to differences in skill and technique on the part of these observers. Dr. Boeck noted that these variations were due in part also to variation in the distribution of the parasites in the feces.

Dr. Hall presented a note proposing new generic names for the species *Strongylus rubidus* Hassall and Stiles and for *Filaria osleri* Cobbold. He also noted the presence in *St. rubidus* and in *Ornithostrongylus quadriradiatus* (Stevenson) of peculiar accessory structures, which were described by the authors of these species. He found that these structures stain well with gentian violet and as they are very transparent he suggested that other nematodes be examined by means of this stain, as it appears that the structures are much commoner than is indicated by the lack of records regarding them. These structures serve to support the cloacal aperture, the terminal portion of the cloaca, and the genital cone. He proposed the name *telamon* for such a structure. Gentian violet is a very amenable stain which seems well adapted to the study of this structure and of the bursal rays and some other nematode structures.

Dr. Cort presented a note on Prenatal Infestation with Parasitic Worms (Published in the *J. Am. M. Assn.,* 76: 170-171.)

Dr. W. H. Welch made some interesting remarks, calling attention especially to a new synthetic arsenical, tryparsamide, which had been found very

effective against *Tr. gambiense,* although apparently of little value against *Tr. rhodesiense.* In using it on African natives, it was found that the natives raised no objection to intravenous injection, but objected to intramuscular injections.

The forty-eighth meeting of the society was held December 10, 1920.

The following were proposed for active membership: D. L. Augustine, Elery R. Becker and Charles E. Simon, by Dr. Cort; W. G. MacCallum, by Dr. Ransom; and were duly elected.

Dr. Hall presented a note in regard to carbon tetrachloride as an anthelminthic for use in removing hookworms. Tests of this drug on thirty dogs showed that in comparison with similar tests of other substances used to remove hookworms, carbon tetrachloride was much more effective, removing all of the worms with considerable certainty when administered in capsules in doses of 0.3 mil per kilo of weight of dog without purgation. It was given with safety in doses of 1.5 mils per kilo, without the production of evident toxic symptoms or postmortem lesions macroscopically visible. It also removed ascarids, being but slightly less effective than chenopodium for this purpose. Carbon tetrachloride is cheaper than other drugs now in use for removing hookworms. A chemically pure product must be used.

In comment, Dr. Stiles noted that chenopodium and thymol are the only drugs extensively used at the present time. Caius and Mhaskar have recently stated that there are no records of deaths from thymol; Dr. Stiles reported that he knew of about sixteen deaths from this drug. Chenopodium has a death list of about 70, according to a recent writer; Dr. Stiles noted that three deaths had occurred in one day in Kentucky from this drug. Chenopodium is not uniform in its composition, a fact which adds to the danger of using it. The cost of treatment is an important factor in large-scale hookworm campaigns. With any anthelmintic the field worker must run some risk, the risk in his case being greater than that in private practice or in hospital work, but the risk is offset by the actual and potential benefit. Carbon tetrachloride, if found satisfactory in human medicine, would have the advantage of being cheap of itself and saving the cost of purgation.

In further comment, Dr. Cort noted that there had been some rioting in Ceylon, following deaths from chenopodium poisoning.

Dr. Stiles presented the following charts covering effects of temperature on hookworm eggs and larvae and on fly eggs and larvae:

Condensed Hookworm Thermometer

8 to 10° C. (46.4 to 50° F.): This is the lowest demonstrated temperature at which hookworm eggs, placed under favorable conditions, will segment and will hatch out larvae that reach the infecting stage.

8 to 18° C. (46.4 to 64.4° F.): In this range of temperature, hookworm larvae are sluggish to motionless.

20 to 35° C. (68 to 95° F.): Favorable to hookworm development and motility.

25 to 30° C. (77 to 86° F.): Optimum for development of hookworm eggs and larvae and for motility of larvae.

35 to 40° C. (95 to 104° F.): Less favorable to hookworm development and motility.

40 to 50° C. (104 to 122° F.): Eggs have been observed to hatch at 40° C., but in general constant temperatures above 37° C. are reported as unfavorable to fatal for eggs and larvae. However, both eggs and larvae can stand 40 to 50° C. for a few minutes and survive.

50 to 60° C. (122 to 140° F.): Fatal to eggs and larvae in 1 to 5 minutes.

Above 60° C. (above 140° F.): Fatal to eggs and larvae almost instantly.

The data for a house-fly thermometer do not seem to be so extensive as in the case of hookworms, but the following points quoted by Howard (1911), are of distinctly practical importance.

Condensed House-Fly Thermometer

7.2° C. (45° F.): Eggs of *Musca domestica* did not develop until brought into a warmer temperature.
12.2° C. (54° F.): Larvae had not matured at end of 8 weeks.
15.6° C. (60° F.): Eggs have been hatched in 12 hours.
18.3 to 23.9° C. (65 to 75° F.): Duration of life round was 3 weeks.
23.9 to 26.7° C. (75 to 80° F.): Eggs have been hatched in 8 to 12 hours.
32.2 to 36.7° C. (90 to 98° F.): Larvae mature in shortest period in fermenting materials.
37.8 to 43.3° C. (100 to 110° F.): Larvae leave the hotter portion of manure.

These charts indicate the temperatures under which sanitation is most urgent and those under which sanitation becomes a matter of less importance and urgency. Above and below the favorable temperatures, compromise measures in sanitation become possible. If feces can be pasteurized, hookworms may be killed in this manner. Diet is an important factor in this connection. Where much meat is eaten, as in northern climates and in city workers, the feces offer less favorable conditions for hookworm development than where little meat is eaten, as in southern climates and in rural districts.

In an additional note, Dr. Stiles stated that ground water pollution must be regarded as a standard in sanitation. This is true as regards the storage and disposal of excreta. Dr. Cox in Virginia has found coal gas tar and kerosene of value in preventing fly breeding. Working independently, Dr. Stiles, at a later date, tested coal gas tar and found its use a very cheap and effective control measure for flies. The tar costs 5 to 20 cents a gallon, depending on whether it is purchased in car load lots or by the gallon. Added to feces it does away with the necessity for screening against flies. Since dark privies attract anopheline mosquitoes, malaria carriers, light privies may be used with the tar without attracting flies, as light privies do. The mixture of tar and excreta may be ultimately dumped and covered with sawdust, without subsequent fly breeding. A gallon of coal tar to a cubic yard of soil does not appear to inhibit the growth of vegetation.

In comment, Dr. Hall noted that empty tar barrels standing in the hot sun must be regarded as possibly dangerous, as a newspaper has published one instance in which throwing a lighted match in an empty barrel of this sort caused an explosion with the death of one person resulting.

Dr. Cort reported that the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, would undertake investigations in Trinidad under the auspices of the Rockefeller Foundation in regard to hookworm larvae in soil and the factors of importance in this connection. This work will be carried on in the summer of 1921 in collaboration with Dr. Payne of Trinidad, the party from the United States to consist of Drs. Cort and Ackert and Mr. Augustine.

Mr. Chapin reported the occurrence of *Dipylidium sexcoronatum* in the cat, the specimen being collected at New Haven, Connecticut.

Dr. Schwartz presented the following note:

SUMMARY OF LITERATURE ON EFFECTS OF EXTRACTS OF PARASITES ON PATHOGENIC BACTERIA

André (1878) reports that Taenia infestation in cases of pulmonary tuberculosis in man has a beneficial effect on the patient by retarding the course of the disease. Granger (1897) corroborated this view on the basis of his clinical findings.

Picou and Ramond (1889) found that extracts of Taenia inhibit the growth of various pathogenic microorganisms in vitro. These writers also state that injections of Taenia extracts into guinea-pigs rendered them more or less refractory to infections with the cholera organism, since nine out of ten guinea-pigs thus treated survived. Jammes and Mandoul (1904) failed to find bactericidal properties in Ascaris fluid. They also found that many pathogenic

bacteria were unaffected by extracts of *Taenia pisiformis*. They found, however, that extracts of *Moniezia expansa* retard the development of certain pathogenic bacteria *in vitro*. Jammes and Mandoul also state that injections of extracts of Taenia and tubercle bacilli give rise to a slower development of tuberculosis with resultant milder lesions than the injection of tubercle bacilli without Taenia extract.

Joyeux (1906) found that in a general way extracts of nematodes are not bactericidal but that extracts of cestodes possess bactericidal properties.

Perroncito (1912) failed to confirm the results of previous investigators concerning the retarding influence of Taenia extracts on the development of tuberculosis in guinea-pigs. He found that guinea-pigs injected with *Bacillus tuberculosis* developed as intense lesions as guinea-pigs injected with tubercle bacilli alone. Perroncito found, however, that dysentery bacilli (Flexner and Shiga) do not develop in Ascaris fluid. Like wise, he found that extracts of species of Anoplocephala from the horse are bactericidal to dysentery organisms (Shiga type).

Alessandrini (1912) found that extracts of several species of cestodes and nematodes retard the development of certain pathogenic bacteria in vitro. Anthrax organisms showed the greatest susceptibility to these extracts. Alessandrini also found that the administration of Ascaris fluid and *Bacillus pyocyaneus* into dogs *per os* brought about the disappearance of the bacteria since they could not be recovered in the feces. Control dogs fed *Bacillus pyocyaneus* showed the organisms in the feces. He obtained similar results in dogs harboring *Taenia pisiformis*, showing that this parasite is detrimental to *Bacillus pyocyaneus*. This writer found moreover, that chickens harboring *Heterakis papillosa* were practically immune to fowl cholera.

Perard (1912) examined post mortem 300 tubercular cattle and 300 non-tubercular cattle with a view of determining the incidence of tapeworm infection complicated by tuberculosis. He reached the following conclusions:

1. Tuberculosis is found in the same frequency in cattle infested with tapeworm as in cattle free from tapeworms.

2. Tubercular lesions are frequently found in the neighborhood of tapeworm lesions.

3. There are as many advanced cases of tuberculosis and tapeworm infestation in cattle as there are cases of incipient tuberculosis and tapeworms.

Despite the contradictory evidence, it must be admitted that the published data warrant the belief that certain parasitic worms contain bactericidal substances. The bactericidal substances are specific for certain microorganisms and do not appear to influence the growth and development of other organisms.

The writer found that physiological salt solution extracts of *Ascaris lumbricoides* inhibit to a considerable extent the growth of *Bacillus pyocyaneus* in vivo and in vitro. One series of experiments on guinea-pigs yielded the following results:

Two guinea-pigs injected subcutaneously with Ascaris extract and *Bacillus pyocyaneus* showed a small localized, non-suppurating abscess at the site of injection. The animals survived. Two control guinea-pigs injected with material from the same culture, without the extract, developed large abscesses which extended over the entire abdomen and which resulted in death of the animals after several weeks.

In connection with the bactericidal properties of parasitic worms, it may be noted that shortly after the rise of bacteriology as a medical science, parasitology lost considerable prestige among medical men. Prior to the discovery that many diseases of man, well known clinically, were caused by specific bacteria, worms were considered by many medical "authorities" as causative agents of various diseases. Relegated from its one time important position, parasitology became a neglected field of medicine. As a matter of fact worms came to be regarded by some writers as beneficial to the host and were said to be "the guardian angels of children." Guiart (1914) considers the views of

Alessandrini (1912) with reference to the bactericidal properties of parasitic worms as an echo of the view of the alleged beneficial rôle of parasites in the economy of the host.

In comment, Dr. Stiles noted that the removal of hookworms from patients suffering from tuberculosis led to improvement in the tuberculosis, and that in some parts of Kentucky the incidence of hookworm disease was said to coincide in general with the greatest incidence of tuberculosis.

Dr. Bartsch exhibited a turbellarian worm (Leptoplana sp.) parasitic on oysters and doing considerable damage to oyster beds in the Gulf of Mexico.

Dr. Cobb presented notes as follows:

A serious disease of the cocoanut palm, due to nematodes, has from time to time appeared in the West Indies and, in certain parts, wiped out the industry. It has now appeared in Panama. The cocoanut palm is a very important plant, its oil content being of such value that it would be a very important competitor with butter, except for its lack of vitamines.

A nematode was reported from the hot springs in the Yellowstone Park, the worm living at a temperature of 53° C.

Results of further study of rhabditin, a substance in nematodes which was previously discussed before the society, were reported. Of eighty nematode genera examined, forty-four showed no birefringent granules, though very small ones might have been overlooked. These granules present in the other thirty-six genera have been named by adding the suffix *in* to the generic name. Sometimes there are six or seven types in one organism, usually in the intestine. They are divided into two classes, one anabolic and one katabolic or excretory, and these classes are indicated by the prefix *ana* and *kata,* thus, anarhabditin and katarhabditin. In the case of such nematode genera as end in *us,* this is dropped, thus, anoncholaimin. Little is known about these substances in parasites. Anarhabditin is of interest, since this substance is used up in reproduction, the granules nearest the genitalia disappearing first. Plasts or shells of the rhabditin are left behind in the cells. The anabolic granules are usually spheroidal or ellipsoidal, while the katabolic granules are sometimes true crystals.

The forty-ninth meeting of the society was held January 29, 1921.

Dr. Cobb presented a note on nematodes collected by the Canadian Arctic Expedition under Stefansson. He has in his possession now nematodes from the North and South Polar region, including land and fresh water forms. The free-living nema collection of the Canadian Expedition is a large one, totaling 7,404 specimens, all of which have been examined. These were mounted on slides and charted under low magnification ($\times$ 5) by means of camera lucida, the chart showing the specimen number and identification, each specimen being distinguished by a record of position, form and size. Of the specimens, 70 per cent. were species of Plectus, and of these 50 per cent. belonged to two species. Many specimens showed evidence of a microzoan disease, and the same is true of Antarctic Plecti. There are a total of twenty-two genera and forty-seven species represented. About 50 per cent. of the species are common much farther south, many occurring in the vicinity of Washington, D. C., and in the tropics, and such species must be regarded as truly cosmopolitan.

This material afforded an opportunity to make a study in measurements. Fully mature, perfect specimens, perfectly preserved and exactly in profile, when carefully measured showed as much as a 50 per cent. variation in actual length while the ratios utilized in nematode formulas were quite definite and constant.

In the discussion, Dr. Bartsch noted the probable importance of wild fowl as carriers of free living nemas, reporting that molluscs showing certain aberrations occasionally appeared in various localities and then died out, probably having been carried in on the feet of wild fowl, developing in the new and

unsuitable environment in an abnormal manner, and then dying out. In newly made ponds or lakes, the mollusc fauna of bodies of water some distance away has been seen to appear very soon, probably being brought in on the feet of various water birds.

With reference to variations after maturity in parasitic worms, Dr. Cort noted that schistosomes may begin egg-laying, indicating maturity, and subsequently grow three or four times as large. He also raised the question as to whether a parasitic species does vary considerably with different host species. Dr. Ransom sustained the contention that a worm species does vary with the host species, pointing out that *Syngamus trachealis* attains a length of 20 to 25 mm. in the chicken and 40 to 45 mm. in turkeys.

Dr. Bartsch reported on a number of molluscs obtained from dealers in aquarium supplies sent him by Dr. Stiles for determination with reference to the possibility of the introduction of fluke diseases through the importation of snails for use in aquariums. Two of the forms examined are Japanese, Planorbis and Vivipara, a third species being an Ampullaria, evidently American, as it has a horny operculum, the operculum in Asiatic species being calcareous.

Dr. Bartsch also noted as of interest to helminthologists in view of the historical association of shipworms with the subject of helminthology, that shipworms on the West coast of the United States have cost the United States navy at least $25,000,000. A matter of interest is that no European species have become established in the United States so far as known, probably because the era of wooden ships ended before these worms could become established and also because of essential differences in environment. Species are sharply restricted in distribution by their requirements in respect to fresh, brackish or salt water.

Dr. Ransom presented a preliminary report by Dr. Ransom and Miss Cram on the course of migration of ascarid larvae in the body. Stewart suggested that the larvae probably went by way of the portal system to the liver and thence by the hepatic veins and vena cava to the heart and pulmonary arteries to the lungs, but possibly by way of the gall duct to the liver and thence to the heart and lungs. Ransom and Foster regarded the route by way of the liver as the probable one. Yoshida reported that larvae went to the abdominal cavity and thence to the lungs and elsewhere by penetration of tissue, migration by way of the blood vessels being considered of minor importance. Ransom and Cram are unable to confirm Yoshida's findings. They find the larvae in large numbers in the circulatory system, including the portal vein, vena cava and right side of the heart. In sections of the liver from animals infected the day before, the larvae are often found in the blood vessels but never in the biliary canals. In less than twenty-four hours they have been found in the superficial lymph nodes, including the submental nodes. In three they have been found in the inguinal, axillary and deep cervical lymph nodes and in fact during the first week they have been found in practically all the lymphatic nodes. Within twenty-four hours they can be found in the mesenteric lymph nodes in large numbers, especially in the ileocolic nodes. It appears from these findings that the blood and lymph streams constitute the important paths of migration.

Dr. Cort called attention to the fact that Tanabe has reported *Echinostoma perfoliatum* var. *japonicum* from man. Encysted stages of this worm were first discovered in fresh water fish and fed to dogs, producing the adult worms. Subsequently echinostome eggs were found in human feces, and it was surmised that these came from worms of the same species. To settle this point, Tanabe ate some of the encysted forms from fish and developed in himself worms giving rise to eggs apparently identical with those found in feces in the first instance.

Dr. Cort also presented a summary of our knowledge in regard to the course taken by the larvae of *Schistosoma japonicum* from their entrance by way of the skin to their final destination in the blood vessels. This point has been contested at some length by Japanese workers. Miyagawa has examined

dogs to ascertain the course of the larvae and concludes that the larvae go by way of the venous or lymphatic systems to the heart and lungs, thence to the arteries of the liver and intestine, and thence to the portal system, passing two sets of capillaries. Narabayashi infected young mice and sectioned them *in toto* and concludes that the worms go to the heart by way of the venous system and, in smaller numbers, by way of the lymphatic system, some of them dying in the lymph nodes; from the heart they go to the lungs, and from here they proceed in small numbers along the path indicated by Miyagawa, but much the larger number of larvae pass from the lungs to the pleural cavity and mediastinum, migrate through the loose connective tissues to the liver and thence into the portal system. Narabayashi's conclusions are confirmed by another Japanese worker. The schistosome is a blood voyager and grows but little until it reaches the liver, whereas Paragonimus is a tissue and cavity migrator, traveling slowly and growing rapidly as it migrates, and apparently maturing about the time it reaches the lungs.

Dr. Tubangui noted that an Echinochasmus is present in the dog in China.

Mr. Chapin exhibited a specimen of a tapeworm from the whale, *Balaenoptera musculis.* This worm has a very odd unarmed scolex. The scolex in the unattached worm is cylindrical, but after insertion in the intestinal mucosa the scolex becomes spherical, thereby attaching the worm to the mucosa by a riveting action.

Dr. Stiles presented the following note:

A THIRD CASE OF GONGYLONEMA FROM MAN

Up to the present time, two cases of Gongylonema in man have been reported. The first case was reported by Ward, the worm occurring in the lower lip of a girl in Arkansas. Ward suggested that the worm might be *G. pulchrum.* The second case was reported by Stiles, the worm occurring in the lower lip of a girl in Florida. The present case, the third, involves the occurrence of the worm in the mouth of a girl in Georgia. The worm in this case is badly macerated, but a part of the cuticle is intact near the head and this shows the presence of two of the characteristic bosses. The specimen is a female, 35 mm. long and with the uterus post-equatorial. Almost all of the anterior cuticle is lost by maceration. In the first two cases recorded, the presence of the worm was accompanied by pronounced irritability on the part of the patients. This finding, in connection with the previous records, raises the question as to whether it is a parasite of some animal other than man which is accidentally present at times as a parasite of man, or whether it is a parasite of man now being found but previously overlooked. For the purpose of scientific record it is better to keep a doubtful species distinct, even though it is later necessary to suppress a name as a synonym than to take the chance of confusing two things under one name, with the more serious difficulty of disentangling biological facts. For the Gongylonema from man the name *Gongylonema hominis,* species inquierenda, was proposed with Ward's specimen as type material.

Dr. Stiles also reported that in experiments on gas house tar it had been found that the tar could be set on fire by the use of paper and that precautions were being taken, in connection with the use of this substance to prevent fly breeding in feces, to safeguard against fire in its use.

Dr. Hall presented the following notes:

A NEMATODE WITH SIX UTERI

A nematode collected from the mesenteries of the puff adder, *Bitis arietans,* by Dr. H. H. Curson of Grahamstown, South Africa, was found to have six uteri. On comparison with the descriptions in a recent paper by Baylis, this material appears to be *Polydelphis quadricornis.* Baylis lists a total of four species of Polydelphis as having six uteri, which is the maximum known among the nematodes.

FILARIA NYCTICEBI MOENNIG, 1920

A parasite recently described from *Nycticebus tardigradus* in the Centralblatt Bakt., Parasitenkunde, etc., is evidently a Rictularia, as the description and figures clearly show. To avoid confusion in the consideration of species of Filaria and of Rictularia, the species is here transferred to Rictularia as *R. nycticebi* (Moennig, 1920).

GID IN SHEEP IN COLORADO

Under date of January 13, 1921, Dr. W. E. Howe reports the occurrence of gid in sheep at Calhan, Colo., a coenurus from the brain of one animal being sent in to the Bureau of Animal Industry at Washington, D. C. This disease has been reported previously from Colorado by Newsom and has twice been diagnosed as present in that state on somewhat uncertain symptoms by the veterinary editor of the Sheep Breeder's Gazette. The history of these cases indicates that the infection originated in Montana, where gid has been enzootic for over thirty years.

STEPHANURUS DENTATUS AS A PARASITE OF CATTLE

Two worms collected from the small bile ducts of a bovine (whether steer, cow, calf or bull not stated) by Dr. T. B. Pote at an abattoir in St. Louis, Mo., were sent in to the Zoological Division of the Bureau of Animal Industry by Dr. J. J. Brougham on January 15, 1921, with a report to the effect that they appeared to be the common kidney worm of swine. This identification was confirmed. The worms were both males of *Stephanurus dentatus*. This parasite has heretofore been reported only from swine. The life history according to Bernard and Bauche is as follows: The eggs from female worms in the perirenal fat enter the ureters through fistulous tracts as a rule and pass to the exterior in the urine. The young worms hatch and develop to the infective stage. In this stage they may enter the host animal by way of the skin or the mouth. In the first case, evidently the normal mode of infection, the worms develop in the vicinity of the kidneys. In the second case the worms develop in the liver, as a rule, though they have been found in various other organs. Apparently therefore the infection, in the case of the animal reported here, took place by way of the mouth.

The fiftieth meeting of the society was held February 26, 1921.

Mr. Ackert presented the following note:

THE LONGEVITY OF FOWL TAPEWORMS

It is known that when certain cold-blooded vertebrates are kept in confinement they quickly lose their cestodes. Opportunity to ascertain whether this phenomenon occurs in certain warm-blooded vertebrates was afforded during the last two years in connection with studies on the life histories of chicken cestodes. The chickens under observations were hatched on farms near Manhattan, Kan., and had free range of the premises. When slightly over four months of age, they were passing cestode proglottids. At this time they were placed in a small third-story room and given food free from animal tissues except an occasional feeding of fresh beef. In midwinter they were transferred to an equally small screened pen with cement floor.

The subsequent intestinal examinations yielded the following results: Chick 324 retained a heavy infestation with *Davainea cesticillus* for four months. Chick 287 showed a heavy infestation with *D. tetragona* and *D. echinobothrida* after five months. Chick 286 had an infestation with forty-eight *D. tetragona* and *D. echinobothrida,* and two *D. cesticillus* after six months. Chicks 287 and 268 had lost all of their tapeworms in eight months. Chick 269 retained 318 tapeworms for eight months, the species represented being *D. tetragona, D. echinobothrida* and *D. cesticillus*. Chick 315 had thirty-eight *D. cesticillus*

and one *D. tetragona* after thirteen and a half months. Chick 284 had lost all of its cestodes in fourteen months. Chick 283 had six *D. echinobothrida* and Chick 285 two *D. echinobothrida* after fourteen and a half months.

In summarizing it is seen that of ten infested chickens placed in close confinement, three lost their cestodes in from eight to fourteen months, and seven retained tapeworms during periods ranging from four to fourteen and a half months. The species of cestodes retained included *D. cesticillus, D. tetragona* and *D. echinobothrida.*

In comment, Dr. Hall noted that an amphistome had recently been reported from a zebra in the London Zoological Gardens under circumstances indicating that worms of the species in question might live at least eight years.

Dr. Cobb raised the question as to the exact action of the hooks on tapeworm heads. In reply, Dr. Stiles stated that these hooks may be observed to work together in a coordinated fashion or in an irregular and incoordinated fashion, each hook apparently acting individually.

Dr. Cobb exhibited some pieces of apparatus for examining specimens in liquids, the apparatus being designed to orient the specimens, especially nematode worms, for observation from different points. Mounted stage forceps were attached to different types of tanks in such a way as to permit of rotating the object in all planes.

Dr. Tubangui presented an abstract of a paper describing a new fluke, a species of Prohemistomum, from the dog in China. This fluke is 1.5 to 2 mm. long by about 1 mm. wide. The genitalia are similar to *Pr. appendiculatum,* but there is no distinct attaching disk.

Dr. Tubangui also gave a discussion, illustrated by figures, in regard to the genus Opisthorchis and related genera. Blanchard listed over twenty species of Opisthorchis. Looss made the genera Metorchis and Holometra and associated them with Opisthorchis in the subfamily Opisthorchiinae. Later the genera Clonorchis, Amphimerus and Paropisthorchis were established. Clonorchis was based on the branching testes, Amphimerus on the separation of the vitellaria into anterior and posterior portions, and Paropisthorchis on the presence of an acetabulum borne on a papilla. Notaulus was based on the presence of testes filling the entire width of the posterior body. Lühe created the subfamily Clonorchiinae for forms with a dorsal excretory pore. All of these genera, except Opisthorchis, are open to criticism. The species left in this genera may be divided into two groups. One group, represented by *O. simulans,* has follicular, much-lobed ovaries, the vitellaria extending from the middle of the body to the ovaries. The other group, represented by *O. felineus,* has smoother lobed ovaries and the vitellaria are posterior to the acetabulum or ovary. But intermediate forms, as regards these features, are found. Species of Amphimerus may have an acetabulum borne on a papilla, as in Paropisthorchis. *O. obsequens* has branched testes and might be considered either an Opisthorchis or a Clonorchis. Similar objections may be raised to the feature forming the chief character of the genus Amphimerus.

Dr. Stiles suggested the possibility of determining variation within a species by mass infection from a single parent parasite.

Dr. Barlow noted that there was considerable difference between specimens of *Fasciolopsis buski* collected from a 9-year-old child and those from a man. Egg production may begin in a specimen 8 to 9 mm. long from a child, while in specimens of the same size collected from adults no uterine structure is visible.

Dr. Bartsch noted that mass studies on snails had shown no variation in species he had thus investigated, the species appearing as if cut in a die, whereas other species are very variable. Species transplanted from the Bahamas could not be crossed on very similar species in Florida under very similar environments, whereas the Bahama species could be crossed on other species in the Bahamas apparently quite dissimilar and unrelated.

Dr. Cort stated that the variations in Fasciolopsis could be largely explained by differences in technique, most of the variations being those of size and shape.

Dr. Barlow noted that these changes were produced by the live fluke in movement and by the effect of fixing fluids, the changes produced by movement while alive not being present in preserved specimens. The fluke with spines anteriorly but not posteriorly is produced by the administration of B-naphthol as an anthelmintic, the posterior part being killed and deprived of its spines.

Dr. Cobb noted that different effects are produced in the appearance of worms by killing with heat and by killing through effects on the nervous system or otherwise.

Dr. Hall noted that worms obtained by anthelmintics were subject to the destructive action of the anthelmintic, carbon tetrachloride being especially destructive to hookworms, and were also subject to the digestive and macerative effects of the intestinal contents. Worms which pass out after long intervals, as in the case of stomach worms of sheep when removed by copper sulphate or ascarids of horses when removed by the use of carbon bisulphide, are usually very much distorted and macerated and often fragmented.

Dr. Barlow noted that B-naphthol frequently produces holes, having the appearance of being punched out, in specimens of Fasciolopsis.

Dr. Stiles noted that if man were studied and described on the basis of variations accepted as satisfactory in the case of flukes, the human group could be separated into a large number of species, if not of genera.

Mr. Chapin reported the findings in a postmortem examination of an African wolf, *Lycaon pictus*. Headless chains of *Taenia pisiformis* were found in the stomach. Fifty specimens of *T. pisiformis,* twelve heads and twenty gravid segments of a worm very similar to the adult *Echinococcus granulosus,* and one Toxascaris, apparently *T. limbata,* were found in the small intestine. The Echinococcus does not appear to be the common species, *E. granulosus.* It is larger and has a head and five segments. Two complete worms were found to measure each 5.04 mm. in length. Lindenfeld has reported a form from the dingo which is 10 to 30 mm. long, and Johnston has noted that this is either a new species or represents the young strobilla of a species other than *E. granulosus.* The hooks of the species from the wolf differ in size and shape from those of *E. granulosus.* A comparison was also made with *Taenia oligarthra.*

Dr. Stiles reported two new cases of *Diphyllobothrium latum,* one from Florida and one from Connecticut. No history of the cases known.

Dr. Stiles also noted that the use of sawdust for admixture with night soil had been legally recognized in some community in its ordinances, this recognition antedating the development of the use of this substance in his investigations. In comment, Dr. Barlow stated that sawdust had been used in China for centuries for mixing with night soil.

Dr. Barlow presented a very interesting note on what he terms fasciolopsiniasis, noting its occurrence in China with special reference to Che-Kiang province and defining it in part as a subacute or chronic disease associated with the presence of Fasciolopsis and resulting in asthenia and death, probably as a result of intoxication. The fluke is the largest of the human flukes and may be 104 mm. long by 18 mm. wide. The flukes may occur from the stomach to the colon, and are sometimes vomited. They attach to the mucosa and patients complain that they can feel the movement of these parasites. Oil of turpentine is used by the natives as an anthelmintic to remove them. B-naphthol has proved the most satisfactory remedy, used in doses of 60 to 75 grains, divided into three portions. There is a marked pallor in this disease, but apparently no true anemia. There are two types of the disease, one with and one without edema. Diarrhea and indigestion are prominent symptoms. There are about one and one-half million cases in the endemic area. Whole families are sometimes wiped out by the disease. The prophylaxis is apparently too general to impress the natives. One native treatment consists in burning the skin with punk. Numerous statistics were given showing how very severe the infesta-

tion may be and how serious this disease is. The talk concluded with the exhibition of photographs and specimens, one of the exhibits being an artificial tapeworm made by stringing these flukes end to end on a thread to form a string 100 feet long, the flukes in this chain being passed by one patient after a single treatment.

Dr. Hall presented the following note:

CODIOSTOMUM STRUTHIONIS FROM STRUTHIO CAMELUS

Under the name *Sclerostomum struthionis*, Horst in 1885 described a worm belonging in the Strongylidae from the ceca of *Struthio molybdophanes*, the ostrich of Northern Africa. This worm was placed by Railliet and Henry in 1911 in the new genus Codiostomum as the type species, and is as yet the only species of the genus. Recently specimens of this worm were forwarded for identification to the Zoological Division of the United States Bureau of Animal Industry by Dr. H. H. Curson of Grahamstown, South Africa, with a label to the effect that they were collected from the stomach of *Struthio camelus*, the ostrich of South Africa. This worm can not be confused with *Strongylus douglasi* Cobbold, 1882, emend. Gedoelst, 1911, which has been referred to the genus Trichostrongylus by Theiler and Robertson in 1915. *Trichostrongylus douglasi* is a very small form with the small trichostrongyle head, without buccal capsule; *Cod. struthionis* is a much larger form with a well developed buccal capsule and corona radiata.

MAURICE C. HALL, Secretary.

BOOK REVIEWS

THE AMERICAN JOURNAL OF HYGIENE. Edited by William H. Welch, with the assistance of a corps of scientific collaborators. Vol. 1, No. 1, Baltimore, January, 1921.

The appearance of the first number of this new Journal published under the auspices of the School of Hygiene and Public Health at Johns Hopkins University and supported by the DeLamar Fund, calls for more than passing mention. The new Journal is devoted to the publication of papers representing the results of research in the field of Hygiene using the term in its broadest sense. Numbers will appear bi-monthly and constitute an annual volume of about six hundred pages.

In his editorial introduction Dr. Welch emphasizes the increasing activity in this country in departments of public health and the emphasis being laid by them on scientific investigation. He comments further on the scattered sources in which this material has appeared and the lack of opportunity for its publication and expresses the hope that the new Journal may have its share in the promotion and distribution of this new knowledge.

Four out of the five articles in the first number fall in the field of Parasitology. These are: an extensive study on the Development of the Japanese Blood-Fluke in its Final Host, by W. W. Cort, an Experimental Study of the Intracranial Parasitism of the Human Lung Fluke, by Yokogawa and Suyemori, On Relationship of Hookworm Infection to the Health of Men in a United States Army Camp, by Kofoid and Tucker, and finally, Recent Experimental Studies on Yellow Fever, by Noguchi.

All workers in this field will recognize both the value of such a publication and its opportunities in this field. The best wishes of all may surely be extended to the editor and his associates for the future of the publication so auspiciously begun.

ESSENTIALS OF TROPICAL MEDICINE. By Walter E. Masters. Published by William Wood & Company, New York.

An American edition of an English work of recent date, the Essentials of Tropical Medicine, by Walter E. Masters, presents in summary fashion and from the standpoint of the man in the practice of medicine, the essentials of a subject which is rapidly outstripping in range all other specialized branches of medical science. It deserves the designation of a vade-mecum for the student and the busy tropical practitioner, a designation already given it by the author.

The method of organizing the material and the type of treatment accorded it are not attractive to the eye because of the numerous paragraph headings in heavy type and the exceedingly concise sentences which make up the text. At the same time it is easy to see that this treatment brings an enormous amount of material within a narrow compass and sets it out in a way to make the individual phases catch the eye of the worker promptly.

The work covers in successive chapters diseases due to protozoa, bacteria, helminths, venoms, poisons, etc. A wise feature and one that is not ordinarily found in a work of this type is the devotion of the closing chapter to laboratory hints. While these are in part elementary, they will serve adequately the purposes of the worker in the field to refresh his mind if he has forgotten the routine of laboratory procedure, and bring up before him a multitude of minor precautions which in the absence of such instructions are very likely to be overlooked, bringing trouble and often unexplained defeat. The figures, while not abundant, are on the whole very well selected and reasonably well produced. The work will serve a valuable purpose, and is worthy of commendation.

INFECTIONS PARASITAIRES. Tome XIV, Traite de Pathologie Médicale et de Thérapeutique Appliquée. By Neveu-Lemaire, Ameuille, J. Troisier, Paisseau, Gouzien, Abrami, and Ramond. Published by A. Maloine & Sons, Paris.

This recent extensive work in French has brought to the parasitologist and those interested in the field a large amount of valuable information in a form that is at once attractive and thoroughly useful. Parasites are grouped in accordance with the diseases to which they give rise, and following hard upon a brief but good discussion of generalities in parasitic diseases, separate chapters are devoted to Helminthiases, Myiases, Protozooses, and Mycoses. Under each heading a disease producing organism, the etiology, pathology, symptomatology, treatment, and prophylaxis of the disease are discussed in order.

The text is admirably up to the limits of present knowledge and the illustrations are in the main good. The majority of them are re-drawn for this work and are well executed. At some times one finds a little tendency towards too schematic or barren a presentation. This may doubtless be explained on the basis that the authors have sought to represent what the student will actually see under the microscope but this, while in a certain sense true, is not adequate justification for omitting details of structure which could be readily observed on careful study by anyone who had been trained in microscopical work.

The amount of space devoted relatively to the different topics is on the whole well balanced. The chapter on worms falls a little short of what would be useful to workers in this field and the section on protozoa is distinctly deficient in range. On the other hand, in the presentation given the student finds it possible to differentiate between those things which are established and those that stand at present in a more or less uncertain position. However, there are a number of important complaints which have received scanty consideration at the hands of the authors. Probably these limitations in the text are self-imposed by the desire of the authors to find a middle ground between the expressive brevity that leaves the mind in constant doubt as to the exact situation and the monographic ponderousness which conceals the essential facts in a mass of collateral details. If this be the end desired, it has been reasonably well attained for the book will serve practical purposes adequately in most instances.

The appearance of the subject volume on Roundworms in the *Index-Catalogue of Medical and Veterinary Zoology* by C. W. Stiles and A. Hassall, calls for more than passing notice. No publication of equal scope has ever been carried to conclusion, and with the increasing complexities of the literature, no publication will be more genuinely welcomed than this volume in the series dealing as it does with a greatly confused group in which even the expert worker finds it difficult to trace his pathway. With the earlier volumes, this gives to workers in parasitology reasonably complete references to the literature of all groups of parasitic worms and, in conjunction with the author index, is a comprehensive and invaluable aid that should be at the side of every worker in these subjects.

Of course the work will not do away with libraries; in fact it renders them even more necessary for it is in itself only an extensive and highly abbreviated set of references which can be interpreted only with the author index and an adequate library. One must regret that delays incident to the appearance of these publications bring in 1921 a subject index to literature that does not extend beyond 1911.

Especial attention should be called, even at this late date, to the work of Wohlbach on Rocky Mountain Spotted Fever (*Jour. Med. Res.*, 41: 1-197, 21 pl.). The demonstration of a micro-organism named *Dermacentroxenus rickettsi* in

the tissues and eggs of infective ticks, and the undoubted developmental phases present in ticks that were infective and absent in non-infective individuals, together with its occurrence in the lesions characteristic of the disease in man, monkey, rabbit, and guinea pig are evidence of its relation to the disease even though the author was unable to cultivate it. Apart from the morphological data worked out with great care, parasitologists will find of distinct interest the discussions of the relation of the parasite and the disease to Tsutsugamushi disease, discussed in the JOURNAL for December, 1920.

NEW HUMAN PARASITE

Eimeria snijdersi Dobell 1921.—Dr. E. P. Snijders recorded (Parasitol., 12: 427-432, figs. A-D) the discovery at Medan, Sumatra, in the stool of a patient ten years in the tropics oocysts of an Eimeria differing from others previously described from man. Dobell agrees with Snijders that they represent a new species which he describes as follows: Oocyst colorless, spherical, 40-48μ in diameter. Spores fusiform, equally pointed at both ends; length 20-25μ, width in middle 7-8μ. Oocystic residue small, granular. Sporocystic residues in the form of one or two small refractile spheres. No crystalline bodies—like those of *E.oxyspora*—visible at the posterior ends of the sporozoites (Parasitol., 12: 433-436, issued Jan. 10, 1921).

NOTES

Especial attention should be directed to the report of the Committee on the Pedagogics of Medical Zoology and Parasitology published in the Proceedings of the Association of American Medical Colleges (30: 167-176). The Committee was composed of Doctors E. R. Stitt, William H. Park and A. I. Kendall, chairman. The report presents the results of a questionnaire, the analysis and critique of the committee, an ideal program, and the discussion which followed the presentation of the report at the meeting of the Association in Chicago in March, 1920.

Professor von Graff, referred to in the preceding number of the JOURNAL (7: 156), is happily not dead tho he has been compelled to retire from service and is now in a sanitarium.

ERRATA

In THE JOURNAL (September, 1920), Vol. VII, p. 16, line 3 from bottom, for *Leptomonas gracilis* read *Leptomonas bütschlii;* p. 17, line 5 from bottom, for chromated read chromatoid; p. 19, line 13, for *Herpetomonads* read *Herpetomonas;* p. 19, line 29, for artifically read artificially; p. 20, line 5, for *Lacerara* read *Lacerta;* p. 21, line 21, for occur read occurs.

INDEX TO VOLUME VII

The actual dates of issue of Volume VII of The Journal were as follows:
No. 1, September 28, 1920 No. 3, April 20, 1921
No. 2, January 9, 1921 No. 4, July 29, 1921